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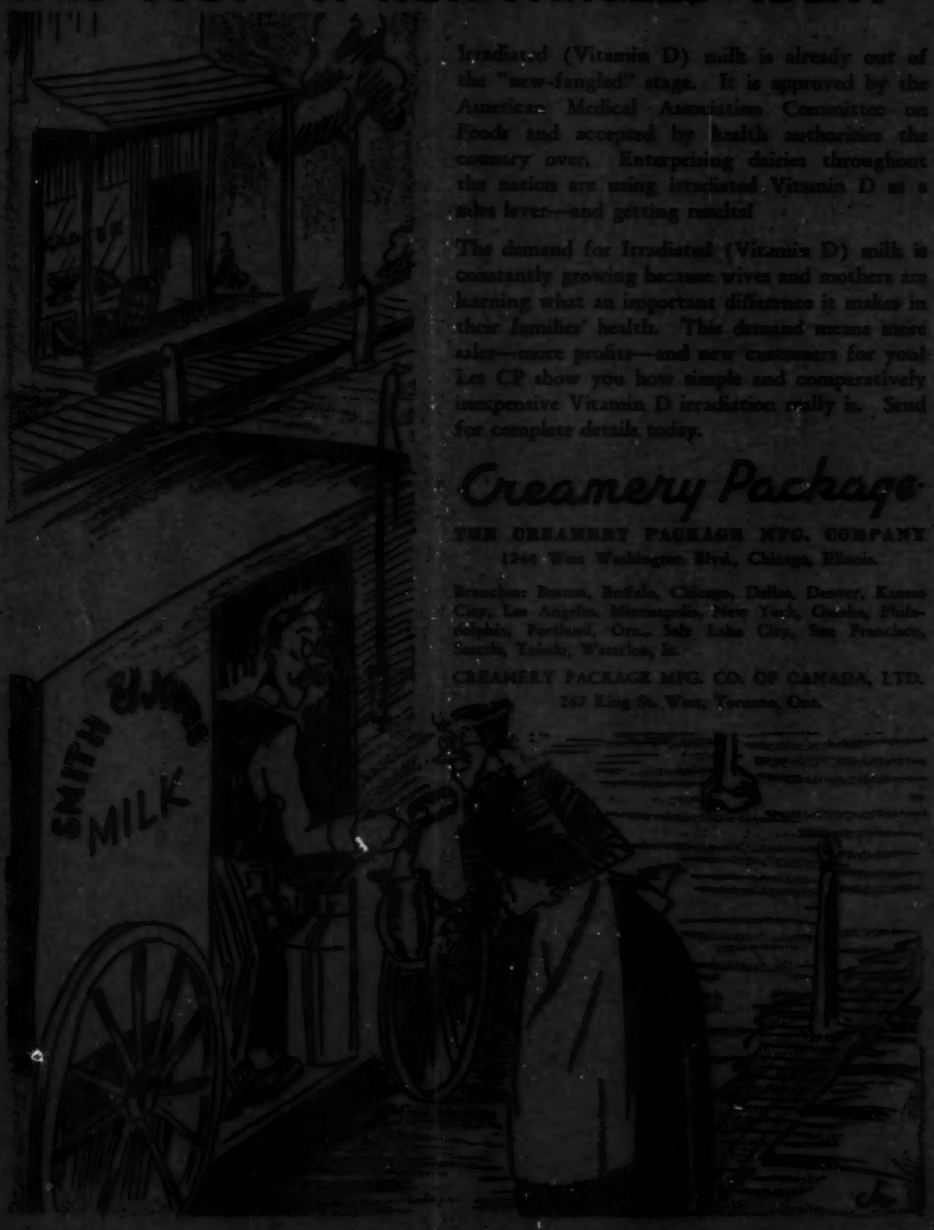

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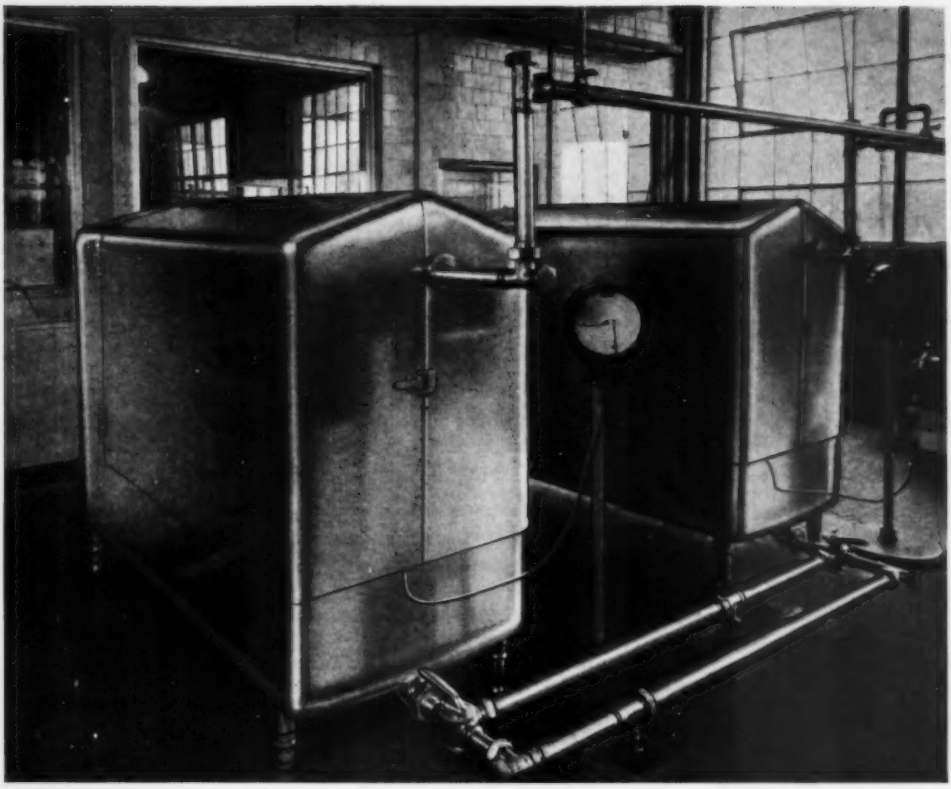

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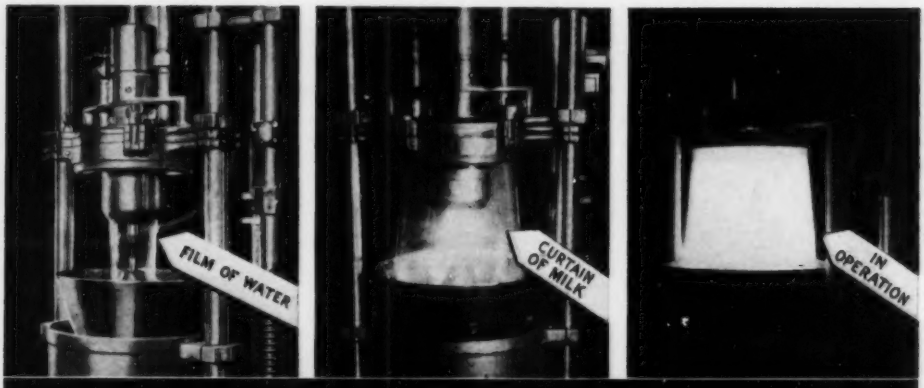

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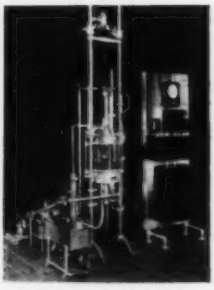

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JOURNAL OF DAIRY SCIENCE

VOLUME XVIII MAY, 1935 NUMBER 5

SOME PHYSICAL EFFECTS OF FREEZING UPON MILK AND CREAM*

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Many observations on the physical effects of freezing upon pure sols have been reported and an interesting review of some of these studies is given by Jones and Gortner (3). An investigation of the effects of freezing upon milk and cream is complicated by the heterogeneous character of the material. The constituents of milk with which this study is chiefly concerned are the casein and the fat.

A hydrophilic sol which has been frozen will repeptize upon thawing whereas a hydrophobic sol similarly treated will precipitate when the frozen mass is melted. The casein of milk, being weakly hydrated retains its normal degree of dispersion under the usual conditions of freezing and thawing. The fact, however, has been noticed that when milk is held in the frozen state for considerable periods of time the casein gradually becomes insoluble (1, 2, 5). Evidently its hydrophilic properties are altered during storage in a frozen condition.

The fat is present in milk as an emulsion and is surrounded by adsorbed protein. If milk or cream has been frozen slowly free fat separates or oils off during thawing, especially when the thawing is conducted at high temperatures. If the product is frozen rapidly enough destruction of the fat emulsion can largely be prevented.

It is to be noted that while a destruction of the colloidal character of the caseinate system during freezing involves a considerable storage period, the effects harmful to the fat emulsion have occurred by the time the material is completely frozen.

A change in the distribution of the constituents when milk is partially frozen has been reported (6, 7, 11, 14). The results show a concentration of constituents in the unfrozen liquid which is to be expected since the solid to separate is pure ice. A condensing process utilizing this principle has

* Presented at the 29th Annual Meeting of the American Dairy Science Association, Geneva, N. Y., June 28, 1934.

Received for publication January 11, 1935.

recently been developed, the milk being concentrated by removing the frozen ice crystals in a centrifuge (10).

An extensive investigation of the effects of freezing upon many physical constants of milk and upon the distribution of the different constituents in the frozen mass has been reported by Cvitl (4). Other papers also present data upon phase distribution (7, 8, 9). In general the results show a concentration of the fat phase in the upper portion of the frozen mass, a higher percentage of protein in the middle or lower portions and the greatest concentration of lactose in the center or last frozen portion.

The effect of freezing upon milk and cream in relation to the marketability of these products has received much attention. A number of investigators have studied the use of frozen cream in ice cream manufacture and detailed directions for the successful handling and storage of frozen cream have been made available. Reports of this work may be found in the trade journals or in Chemical Abstracts.

EXPERIMENTAL

Samples were frozen in air in a cold room maintained at $-16°$ to $-18°$ C. (3.2° to $-0.4°$ F.). Unless otherwise noted the containers used were either tins of 180 cc. capacity or Babcock cream test bottles graduated for 9 gram samples. The material in the tins froze completely in six to seven hours while that in the test bottles required only 50 to 60 minutes to freeze.

The degree of destruction of the fat emulsion in cream was measured as follows: 9 grams of cream were weighed into a 9-gram Babcock test bottle and placed in the $-17°$ C. (1.4° F.) room for 24 hours. The samples were then thawed at 40° C. and water was added to bring the surface of the mixture near the highest graduation on the bottle. They were next placed in a 40° C. bath for 15 minutes, then whirled in a warm (40° C.) Babcock tester for 30 minutes, removed and held in a cold box (15° C.) overnight. The warming and whirling were repeated next day, after which time the length of the column of clear fat was read in the usual manner. Holding the cream cold between whirlings was found necessary to give a clear fat column. Control tests were always run on the unfrozen samples which generally showed about 0.5 to 1.0 per cent fat separation. These figures were subtracted from the readings obtained on the frozen samples. Correction was thus made for the quantity of fat which the method of testing caused to separate and for the action of homogenization in retarding the rise of very small globules into the neck of the test bottle.

Relative viscosity measurements on the frozen and thawed milks were made at 30° C. with an Ostwald type viscosimeter which measured the time required for 24 cc. of milk to flow through one of three different sized

capillary tubes. The water rate for each of the tubes was ascertained and the results are expressed as relative viscosity.

Coagulation and heat stability tests were conducted by sealing the samples in pyrex test tubes and sterilizing them at 120° C. in a glycerine bath.

SOME EFFECTS OF FREEZING UPON THE DISPERSION OF THE CALCIUM CASEINATE SYSTEM

Reference has been made to the gradual change of the calcium caseinate to an insoluble form when milk is held frozen. During this work a progressive decrease in the dispersed state of the casein was produced by freezing.

The effect of freezing upon the heat stability of skim milk was investigated. Data from a representative experiment are given in table 1. Skim

TABLE 1

Effect of freezing at −18° C. (0.4° F.) upon the heat stability of skim milk. Freezing time 6½ hours in cans)

TIME FROZEN	TIME OF COAGULATION AT 120° C.	
	9% S. N. F.	18% S. N. F.
Weeks	Min.	Min.
Not frozen	204	102
7	215	105*
12	225*	95**
17	235**	0
33	205	

* First distinct separation of casein.

** Clear serum could be removed by thawing the frozen mass on a filter at room temperature.

milk of 9 per cent solids was unchanged in heat stability after storage for 33 weeks in a frozen condition. All the samples of this milk were used after the termination of this time, but from the appearance of the milk it was believed that its stability would have dropped to zero in a few more weeks. The milk of 18 per cent solids did not possess any stability toward heat on the 17th week.

Visible separation of the casein in both 9 per cent and 18 per cent milks began to take place early in the storage period. When the precipitated casein first appeared it redispersed during heating but as the time of storage increased more and more casein remained in an insoluble state during sterilization. The precipitated casein did not at first appear to affect the stability of the remaining colloidal particles. Separation of the casein

finally became very marked and if the frozen mass was allowed to melt on a filter, a clear filtrate could be obtained. The calcium caseinate from the 9 per cent milk which remained on the filter when the 12-week sample was thawed possessed remarkable heat stability. Mixed with water in place of serum, its heat stability was 195 minutes; and when the serum from the 18 per cent solids sample was used as a continuous phase, the calcium caseinate from the 9 per cent sample showed a stability of 366 minutes.

The effect of freezing upon the dispersed state of casein in skim milk is shown in a different way through some representative data plotted in Fig. 1. Duplicate cans of skim milk were sterilized at 120° C. for different

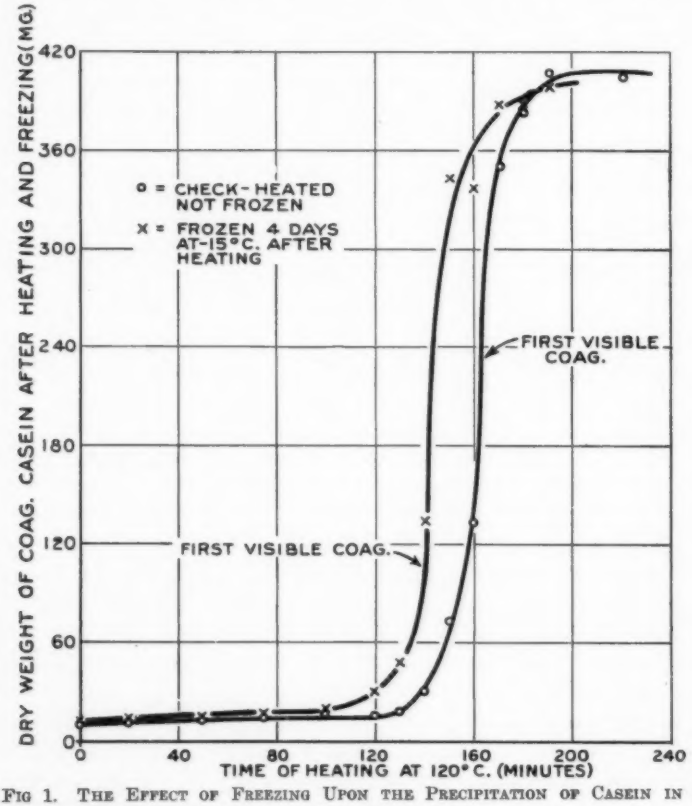

FIG 1. THE EFFECT OF FREEZING UPON THE PRECIPITATION OF CASEIN IN SKIM MILK HEATED TO 120°C. FOR DIFFERENT LENGTHS OF TIME.

periods of time up to 220 minutes. One set of milks was frozen at – 15° C. (5° F.) for four days, the other set acting as the unfrozen control. After thawing the frozen samples at room temperature 10 cc. of milk from each

can was measured into a weighed centrifuge tube. These were centrifuged at a high speed for half a hour, the supernatant liquid decanted and the precipitated casein dried and weighed. The curves of Fig. 1 are of the same type but the freezing process has caused a considerable increase in the amount of heated casein which was found precipitated. The change after a few days freezing is so slight it cannot be detected by visual examination of the sol or by comparing its heat stability before and after freezing. Since this decrease in the degree of dispersion of the casein occurred within a few days after freezing, it indicates that denaturation of the caseinate system during freezing proceeds in a very limited but progressive manner.

SOME EFFECTS OF FREEZING UPON THE FAT EMULSION

While freezing milk over a long period gradually produces an increase in precipitated protein its effect upon the fat phase is much more rapid.

The amount of destruction of the fat emulsion in frozen milk and cream is dependent upon several variable factors. The most important of these appear to be the freezing point of the aqueous phase, the protection afforded the emulsified fat by adsorbed protein and the size of the globules themselves.

The proportion of fat "oiling off" or separating from frozen creams of different fat percentages was measured by means of the test previously described. Large differences in the amount of fat separating after freezing were found. Generally about 25 to 50 per cent of the fat present in 20 to 40 per cent cream and 40 to 60 per cent or more of the fat present in creams over 40 per cent was freed from its normal emulsoid state by freezing. Different creams of the same fat content often showed considerable differences in the amount of fat which separated. In rare instances a cream sample was obtained in which the fat globules were apparently very well protected since only 2 or 3 per cent fat could be separated by the above test after freezing.

Two methods were employed to lower the freezing point of cream, one adding cane sugar and the other increasing the milk-solids-not-fat. Cane sugar in quantities ranging from 5 to 25 per cent added to cream before freezing was found to retard markedly the oiling off of the fat after thawing. The larger quantities of sugar almost entirely inhibited fat separation.

An increase in percentage of milk-solids-not-fat retarded the destruction of the fat emulsion in cream and a decrease in solids favored fat separation. Representative data are presented in table 2.

As the percentage of solids-not-fat of the cream was decreased the test readings for free fat increased from 5.5 to 27 per cent which, on the basis of the total fat present, corresponds to a destruction of the fat emulsion from 12 to 54 per cent. The protective action of high solids can probably

TABLE 2

Effect of variation in solids-not-fat upon the fat separation in frozen and thawed cream thinned from cream containing 60%–63% fat. (Freezing time 60 minutes at –18° C.) Figures represent fat column readings.

MIXTURE NUMBER	THINNING INGREDIENT	COMPOSITION WHEN FROZEN		FAT SEPARATION AFTER THAWING
		Fat	S. N. F.	
		%	%	%
1	Condensed milk	45.5	12.14	5.5
2	Water and condensed milk	46	9.81	10.5
3	Skim milk	45	4.86	15.0
4	Skim milk	40.5	4.05	15.0
5	Water	50	2.62	27.0

be attributed partly to the protection afforded by the protein but chiefly to the lowering of the freezing point caused by the lactose and salts.

The degree of dispersion of the fat slightly influences its capacity to undergo freezing without undue fat separation. The effect of homogenization upon the stability of the fat emulsion was studied. The data of table 3 represent the average figures from three series of experiments. Homog-

TABLE 3

Effect of homogenization upon fat separation in cream during freezing. (Freezing time 60 minutes at –18° C.) Figures represent fat column readings.

FAT IN CREAM	FAT SEPARATION AFTER THAWING		
	Not homogenized	Homogenized at	
		1500 lbs.	3000 lbs.
%	%	%	%
10	1.4	1.1	0.7
20	7.9	5.7	3.6
30	10.5	16.1	18.5
40	25.6	22.2	24.9

enization of 10 per cent and 20 per cent creams helped to decrease fat separation during freezing but in 30 per cent and 40 per cent creams this inhibiting influence was not evident. The amount of clumping caused by homogenization of low fat creams is small but becomes much greater as the fat content of the cream is raised. The clumps are easily broken up by the freezing process and the fat emulsion is destroyed.

The results appear to indicate that the fat emulsion in ice cream remains intact for at least three reasons. The high percentage of cane sugar lowers the freezing point of the aqueous phase; homogenization of the mix inhibits fat separation and increases the amount of protein and gelatin adsorbed; and the formation of countless small ice crystals in the freezer prevent the growth of large and destructive crystals.

Interesting evidence which shows the extent to which freezing destroys the fat clumps in homogenized cream was obtained. Homogenization is known to lower to a striking degree the heat stability of cream. The lowered stability is considered to be due to the formation of fat clumps during homogenization, these clumps acting as nuclei around which coagulation may proceed (13). Data resulting from a study of the heat stability of homogenized creams before and after freezing are presented in table 4.

TABLE 4

Effect of freezing for 24 hrs. at −18° C. upon the heat stability of cream heated to 80° C. and homogenized at 2500 lbs. pressure prior to freezing. (Freezing time 6½ hours in cans.)

FAT	TREATMENT	HEAT STABILITY AT 120° C.	
		Before freezing	After freezing
%		min.	min.
10	Not homog.	122	121
	Homog.	102	120
20	Not homog.	135	137
	Homog.	75	137
30	Not homog.	142	142
	Homog.	2	146

Freezing restores to the cream the heat stability which it possessed before large fat clumps were formed by homogenization. The clumps are apparently disintegrated during freezing and cannot therefore initiate a general flocculation of the caseinate system during heating. Accordingly a homogenized cream which feathered in coffee because of excessive fat clumping could be freed of this defect by freezing. However the free fat which would oil off on the coffee after the addition of frozen cream would be undesirable.

PRACTICAL APPLICATION OF STUDIES ON THE EFFECTS OF FREEZING ON MILK AND CREAM

An application of the foregoing study to manufacturing problems has yielded some interesting data and the possibility of obtaining new products which may be of value either to research workers or to the industry itself.

A concentrated frozen milk (12) may be produced by pasteurizing fresh whole milk, condensing it to one-third of its weight, sealing it in containers and subsequently freezing it in an ice box maintained at about −17° C. Such a milk, if held at a temperature below −13° C. (8.6° F.) may be thawed and easily reconstituted with cold water. It will yield a normal fresh milk at any time up to the 4th week of storage. This product is an excellent substitute for market milk where the latter is expensive or not easily available. It is essential that the milk be handled in equipment constructed of metals other than copper since contamination with this metal will cause a metallic flavor to develop in the product after a storage period of approximately one week.

The usual fat separation which occurs during the thawing of slowly frozen whole milk does not take place in milk condensed to a 3:1 ratio before freezing. The high solids-not-fat content of the product prevents fat separation in the milk just as it does in the creams referred to in table 2.

The denaturation of the casein during freezing is more readily noticed when the solids-not-fat content of the milk is raised. If the milk is condensed to less than one half its weight, the casein concentration is sufficiently great to produce a gel structure as denaturation in the frozen state sets in.

The relation between concentration, fat separation and protein denaturation or gel formation in frozen milks condensed to various degrees is shown by the data given in table 5. A milk evaporated to three times its

TABLE 5

Relation between concentration, fat separation and gel formation in frozen milk. (Freezing time 6½ hours in cans.)

CONCENTRATION NORMAL = $\frac{\text{FAT } 4\%}{\text{SNF } 9\%}$	FAT SEPARATION		TIME TO FORM GEL AT −15° C.
	18 hrs.	2 mos.	
½ N (% fat × 2)	2.0	3.0	No gel. Ppt. in 3 mos.
N	1.0	1.5	"
2 N	trace	trace	3 mos.
3 N	0	0	5 wks.
4 N	0	0	5 days

normal concentration was found to be the most satisfactory for freezing. The data of table 5 may be considerably varied by changing manufacturing conditions and temperatures. High or long heat treatment shortens the storage period in which the milk is free from casein precipitation. High storage temperatures produce the same effect.

The development of viscosity in concentrated frozen milk during storage at two different temperatures is shown by the data plotted in Fig. 2.

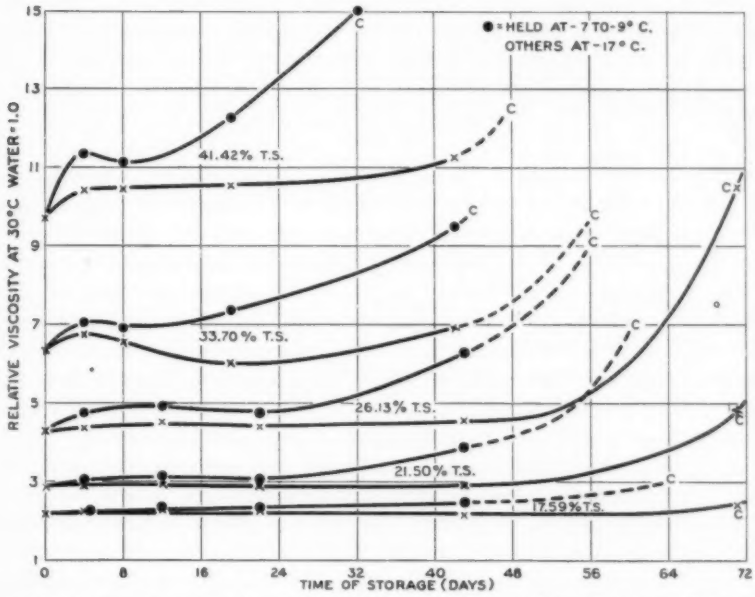

FIG. 2. THE EFFECT OF TIME OF STORAGE IN THE FROZEN STATE UPON THE RELATIVE VISCOSITY OF CONDENSED MILKS OF DIFFERENT CONCENTRATION. "C" AT THE END OF EACH CURVE INDICATES THE TIME WHEN CASEIN COAGULATION WAS FIRST FOUND.

Whole milks condensed to different percentages of solids were used. In most cases when the slight coagulation which precedes gel formation set in, viscosity measurements could no longer be accurately made. In these cases the probable course of the curves is indicated by dotted lines. The importance of low temperatures of storage in preventing the development of high viscosities is apparent.

The ability of the homogenization process to increase protein adsorption and fat clumping in cream was utilized in a method developed to separate some of the milk constituents. It was observed that when homogenized frozen cream was thawed at a temperature below the melting point of the fat, the serum could be drained from the thawing mass, leaving behind a mixture of fat and adsorbed casein. If the melting temperature was high enough to liquefy the fat the material melted as cream and no separation was possible. The fat-casein mixture could be washed with ice water without loss to remove traces of serum.

Data are given in table 6 which are representative of the composition of the fat-casein mixture obtained from creams of 10, 20 and 30 per cent

TABLE 6

Composition of the fat-casein mixture secured from frozen and thawed cream. (Freezing time 10 to 12 hours in 1 gal. cans at −18° C.)

FAT IN ORIGINAL CREAM	WATER	FAT (BABCOCK)	PROTEIN (TOTAL N × 6.38)	LACTOSE AND ASH (BY DIFFERENCE)	CLARITY OF SERUM REMOVED
%	%	%	%	%	
10	53.61	35.0	7.12	4.27	very turbid
20	36.60	55.5	6.59	1.31	turbid
30	20.51	74.0	5.29	.20	clear

fat. A clear separation of serum was obtained only with creams above 25 per cent fat. When the fat content of a cream was lower than this figure there was insufficient fat present to adsorb and hold all the casein. Under such conditions some of the casein escaped with the serum.

The fat-casein mixture was found to be of some practical value. If water was added to replace the serum and the mixture warmed, much of the fat separated by oiling off from the casein. A cream separator removed all but about 0.5 to 1.0 per cent fat. The resulting casein dispersion appeared to possess all of its original characteristics. It was almost tasteless in the absence of the serum and developed very little cooked flavor or brown color after heating to sterilization temperature. Its heat stability remained about the same as that of normal milk. The product provided a normally dispersed casein which should be of value in studies of this protein in its native state. It has been used to advantage in heat stability studies in these laboratories.

The fat-casein mixture was used to raise the milk-protein solids of ice cream mixes without also increasing their lactose content. When the fat for the mix was obtained entirely from frozen cream, the procedure for using only the fat and casein of the frozen cream was very simple. The homogenized frozen cream was thawed upon a fine wire netting suspended over a receiver for the serum, the operation being conducted in an ice box held at 5° to 15° C. (41° to 59° F.). After thawing about 24 hours the fat-casein mixture was added to the mix before pasteurization. For small quantities the most rapid method of removing the serum from the thawed cream was by means of a Büchner funnel using suction. Through use of the fat-casein mixture the protein solids of the mix were increased as much as 1.5 per cent in this manner without increasing the lactose or salts.

Low temperature thawing of homogenized frozen cream provided a simple means of obtaining milk serum in large quantities. By careful handling, a serum equal in clarity to that obtainable by ultra-filtration methods was secured. When the serum was obtained as a by-product in the preparation of the fat-casein mixture for ice cream, it was used to advantage in milk sherbet, giving it a distinctive and pleasing milk flavor.

SUMMARY

1. Slow freezing of milk or cream caused a gradual precipitation of the caseinate system and an immediate destruction of the fat emulsion.

2. Freezing did not alter the heat stability of skim milk until the product had been held frozen for several months at −18° C. (0.4° F.) or below. Freezing caused an immediate increase in the amount of casein which could be centrifuged from milks heated before freezing. Freezing, therefore, caused a slow and gradual increase in the size of the casein aggregates but the change was not noticeable until the freezing period was well advanced.

3. The destruction of the fat emulsion in cream during slow freezing was lessened by adding cane sugar or increasing the solids-not-fat content of the cream before freezing. Homogenization slightly retarded fat separation when low fat creams were frozen. Freezing destroyed the fat clumps formed in cream by homogenization and restored to the cream the heat stability which it possessed before processing.

4. Fresh whole milk was pasteurized, condensed to $\frac{1}{3}$ its weight, canned and frozen without any detrimental effects to the body or flavor of the product. This milk when held frozen at a low temperature and reconstituted at any time within a four-week period by the addition of cold water, yielded a product which often could not be distinguished from fresh market milk. Its use where fresh market milk is expensive or not available was suggested.

5. A process for the preparation of large quantities of normal undenatured casein and of milk serum was developed. Frozen homogenized cream was thawed at a temperature below the melting point of the fat; clear milk serum was collected from the melting mass and the residual mixture of fat and casein was utilized in the preparation of normal casein or to raise the protein solids of ice cream mix.

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THE HEAT STABILITY OF EVAPORATED MILK MADE FROM HARD-CURD MILK, SOFT-CURD MILK AND MILK FROM MASTITIS INFECTED UDDERS*

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In the course of some preliminary studies with soft-curd milk, it was found that the milk samples of low curd tension, after condensing, consistently coagulated at lower temperatures or coagulated sooner at the same temperature than did milk of high curd tension. The soft-curd product was furthermore affected erratically by added salts such as calcium acetate, sodium citrate and sodium carbonate whereas the hard-curd milk was invariably further stabilized by the citrate and carbonate and destabilized by the acetate. These results were at first interpreted as indicating some inherent qualitative difference between soft-curd milk and hard-curd milk but this conclusion was soon abandoned since exhaustive study failed to reveal such differences between the two types of milk (1). It was found, however, that udder infections lower the curd tension of some milk sufficiently to bring it under a soft-curd classification (2). In checking on the samples of milk used for the comparisons mentioned above, it was discovered that the soft-curd milk came from a group of cows, several of which reacted positively to mastitis in one or more udder quarters. It was therefore decided to repeat the comparisons using hard-curd and soft-curd milk from animals free from suspicion of udder infections and milk from animals showing sub-clinical indications of mastitis in one or more quarters as determined by the cell count, the thybromol test, and the precentage of chlorides.

EXPERIMENTAL

Methods

The milk used in these experiments came from selected cows of the college herd. These animals were chosen after several months' observation and were known to produce milk of the desired type. Each lot of milk used for comparison was composited from the complete milkings of from 5 to 10 cows, the individuals contributing to each lot being changed with each trial, although several were included in more than one trial in a different grouping. Considerable care was exercised in ascertaining that each lot of milk was true to the type desired for comparison.

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Each lot of milk was first separated, the resulting skimmilk, only, being used in the experiments. This was forewarmed at various temperatures and concentrated (about 2 to 1) in a small vacuum pan at an evaporating temperature of about 135° F., cooled at once and standardized with distilled water to a milk solids content equivalent to that of the other lots of milk in the trial. Samples were sealed in six ounce tins and sterilized in a pilot sterilizer at a temperature of 240° F. Several trial sterilizer runs were made with each lot of milk until a holding interval was found which produced coagulation in at least one of the three types of milk being compared. Following this, two series of sealed samples were prepared from each of the lots of milk, one series containing increasing increments of M/4 sodium carbonate and the other series, increasing increments of M/4 calcium acetate. These were subjected to sterilization at 240° F. for the predetermined time interval and the relative degree of coagulation noted. In most cases additional sterilizing trials, using different holding intervals, were required with one of the lots of milk, and often with two lots, in order to distinguish accurately the effect of the added salt solutions on the stability. The diluting effect of the salt additions was compensated by additions of distilled water, where necessary, so that the total dilution was the same in all cases.

The "coming up" intervals in the sterilizing trials varied from 8 to 12 minutes but were held constant for every direct comparison of the types of milk.

The procedure outlined was followed in every trial and for each different forewarming temperature where these were varied.

The curd tension determinations were made according to the Hill technique (3) using Monier and Sommer's modification (4) and alcohol numbers were obtained by the method of Doan and Minster (5).

Not all of the data obtained in these experiments are presented due to the inability to condense and summarize them into convenient tables. Typical data are shown in sufficient amount to substantiate the conclusions reached.

Heat Stability without Added Salts

In every trial the condensed milk from animals afflicted with subclinical mastitis (hereafter termed mastitis milk) was found to be less heat stable than either the hard-curd condensed milk or the soft-curd condensed milk. This held true regardless of the forewarming temperature used. The results of eight typical trials are shown in Table 1. The data do not indicate any appreciable difference in the coagulability of hard-curd milk and soft-curd milk and from the further observations made, it is believed there is none. In one trial shown, an apparent difference is evident, sample 16S coagulating to a great degree than 16H. This however

TABLE 1

The Relative Heat Stability of Soft-Curd Milk, Hard-Curd Milk and Milk from Udders Infected with Sub-Clinical Mastitis, After Condensation

TRIAL NO. AND SAMPLE*		CURD TENSION	ALCOHOL NO.	FOREWARMING TEMPERATURE	TOTAL SOLIDS CONTENT	"COMING UP" TIME	TIME HELD AT 240°F.	DEGREE OF COAGULATION+
		grams.	mls.	°F	min.	min.		
6	M	34	.....	170	16.81	8	17	4
	H	62	.....	170	16.86	8	17	0
8	M	29	.....	170	17.90	8	15	1
	H	64	.....	170	17.78	8	15	0
13	M	58	8.8	205	18.09	11	15	3
	H	76	8.5	205	19.08	11	40	0
	S	35	9.2	205	19.02	11	40	0
14	M1	41	8.4	205	17.28	11	15	1
	M2	41	8.4	205	17.28	11	23	4
	H	70	8.5	205	17.66	11	23	0
	S	32	9.5	205	17.24	11	23	0
15	M	46	8.8	205	18.06	11	15	2
	H	74	8.2	205	17.84	11	42	0
	S	34	8.8	205	18.12	11	42	0
16	M	47	8.6	180	17.37	12	30	2
	H	66	8.4	180	18.02	12	40	0
	S	34	9.2	180	17.94	12	40	2
17	M1	49	8.4	205	17.72	12	30	2
	M2	49	8.4	180	17.80	12	30	0
	H1	63	8.8	205	20.30	12	40	1
	H2	63	8.8	180	18.36	12	40	0
	S1	32	9.4	205	18.09	12	40	0
	S2	32	9.4	180	18.92	12	40	2
18	M	53	.....	200	17.67	9½	15	1
	H	105	.....	200	17.81	9½	15	0

* M – Milk from udders affected with sub-clinical mastitis.
H – Milk from normal hard-curd milk.
S – Milk from normal soft-curd milk.

+ 0 = no visible coagulation; 1 = slight coagulation; 2 = moderate coagulation; 3 = prominent coagulation; 4 = pronounced coagulation; 5 = complete coagulation.

was the only comparison showing a definite variation. Some differences are evident in trial 17 but these are partly due to the rather considerable variation in the milk solids content of the samples.

The stability of the soft-curd milk in the fluid state toward alcohol

was greater than either the hard-curd milk or the mastitis milk. This is probably a reflection of the lower casein content of the soft-curd milk. The mastitis milk did not show a consistent difference from the hard-curd milk in this respect.

The data obtained for trial 17 indicate that the high temperature of forewarming (205° F.) stabilized mastitis milk to a lesser degree than did the low temperature (180° F.) This is contrary to the usual effect of varying forewarming temperatures on normal milk. While the data presented are not sufficient to be conclusive on this point other results obtained in the study were confirmatory and the data dealing with the effect of added salts (Table 2) also support the opinion.

Heat Stability with Added Salts

When increasing quantities of sodium carbonate and calcium acetate were added to the condensed milk samples, prepared as previously described, the stability of the hard-curd milk and soft-curd milk was affected for the most part in a normal manner. The carbonate usually increased the resistance toward coagulation while the acetate decreased it. The data presented do not show this effect as well as might be wished but other data and examination of the samples for viscosity indicate it to the satisfaction of the authors. The mastitis milk, however, when forewarmed at the higher temperatures, in the majority of cases reacted oppositely, being destabilized by additions of the carbonate and stabilized by additions of the acetate. At the lower temperatures of forewarming the results with mastitis milk were not so consistent. In the majority of cases stabilization or partial stabilization was obtained with the carbonate and destabilization with the acetate. In frequent cases, however, the results were directly reversed and similar to the effects when the forewarming temperature was high. The transition point appeared to be in the neighborhood of 180° F. forewarming temperature. The results obtained, with the same trials shown in Table 1, are presented in Table 2.

While a few discrepancies in some of the data make direct comparisons uncertain, the results are sufficiently uniform to indicate that mastitis milk has a different so-called "salt-balance" after forewarming and condensing than does normal milk. Apparently it is less stable regardless of forewarming treatment than normal milk and exhibits a decided tendency to be stabilized by calcium when forewarmed at temperatures commonly used for evaporated milk.

Effect of Mixing Mastitis and Normal Milk

Since mastitis milk and normal milk appeared to have ionic maladjustments which might be expected to compensate each other if mixed, some mixtures were compared (in the manner described previously) with normal

TABLE 2

The Effect of Sodium Carbonate and Calcium Acetate Additions on the Relative Heat Stability of Soft-Curd, Hard-Curd and Mastitis Milk after Condensation

TRIAL NO. AND SAMPLE		FOREWARMING TEMPERATURE °F.	DEGREE OF COAGULATION* OBTAINED IN STERILIZING WHEN INDICATED AMOUNTS OF SALTS WERE ADDED TO A 6 OUNCE TIN											
			Control	M/4 Na_2CO_3				M/4 $Ca(C_2H_3O_2)_2$						
			0.0	0.2	0.4	0.6	0.8	0.2	0.4	0.6	0.8	ml. Reagent		
			0.8	0.6	0.4	0.2	0.0	0.6	0.4	0.2	0.0	ml. Water		
6	M	170	4	3	3	3	1	4	4	5	5			
	H	170	0	0	0	0	0	0	0	1	2			
8	M	170	1	0	0	0	0	1	2	3	5			
	H	170	0	0	0	0	0	0	0	0	0			
13	M	205	3	3	4	4	5	2	1	0	0	18.09% Milk Solids		
	H	205	0	0	0	0	0	0	0	0	0	18.09% Milk Solids		
	S	205	0	0	0	0	0	0	0	0	0	19.02% Milk Solids		
14	M1	205	1	2	2	3	3	1	0	0	0	Held 15 Min. @ 240°F.		
	M2	205	4	5	5	5	5	4	3	3	2	Held 23 Min. @ 240°F.		
	H	205	0	0	0	0	0	0	0	0	0	Held 23 Min. @ 240°F.		
	S	205	0	0	0	0	0	0	0	0	0	Held 23 Min. @ 240°F.		
15	M	205	2	3	3	...	4	2	1	0	0	Held 15 Min. @ 240°F.		
	H	205	0	0	0	2	4	0	0	0	1	Held 42 Min. @ 240°F.		
	S	205	0	0	0	0	1	0	0	0	0	Held 42 Min. @ 240°F.		
16	M x	180	2	...	3	4	4	1	0	0	0	Held 30 Min. @ 240°F.		
	H	180	0	0	0	0	0	0	0	0	1	Held 30 Min. @ 240°F.		
	S	180	2	2	0	0	0	2	2	3	3	Held 30 Min. @ 240°F.		
17	M1	205	2	1	3	4	5	2	1	0	0	Held 40 Min. @ 240°F.		
	M2	180	0	0	0	1	2	0	0	0	1	Held 40 Min. @ 240°F.		
	H1 z	205	1	1	2	3	4	0	0	0	0	Held 40 Min. @ 240°F.		
	H2	180	0	0	0	1	1	0	0	0	1	Held 40 Min. @ 240°F.		
	S1	205	0	...	0	0	0	0	...	0	1	Held 40 Min. @ 240°F.		
	S2	180	2	1	0	0	0	...	3	4	5	Held 40 Min. @ 240°F.		
18	M	200	1	1	2	2	2	0	0	0	0			
	H	200	0	0	0	0	0	0	1	2	2			

*M – Milk from udders affected with sub-clinical mastitis.
H – Milk from normal hard-curd milk.
S – Milk from normal soft-curd milk.

+ 0 = no visible coagulation; 1 = slight coagulation; 2 = moderate coagulation; 3 = prominent coagulation; 4 = pronounced coagulation; 5 = complete coagulation.

x – Lower milk solids content than other samples.
z – Higher milk solids content than other samples.

milk with respect to heat stability. The data are so heterogeneous that they cannot be arranged satisfactorily in tabular form and are therefore omitted. The results, however, indicate that the presence of mastitis milk in normal milk invariably destabilizes the normal milk. Considering the situation from the opposite standpoint, normal milk added to mastitis milk improves the stability of the latter and also changes it in such a way that calcium additions no longer stabilize it. It should be appreciated that these trials were in the nature of preliminary studies and further comparisons, more carefully controlled, will be necessary before the effects of mixing mastitis and normal milk on heat stability can be completely appreciated.

DISCUSSION

In attempting to understand or explain the differences in the heat stability of mastitis milk and normal milk, the mineral or salt composition of milk from diseased udders becomes of consequence. Data on this subject are rather meagre but Hashimoto (6), Schrodt (7), Sommer (8) and Rosell (9) have presented figures which can be taken as typical. These are shown in Table 3, together with average values for normal milk. Rosell's data are averages for infected and clean quarters of the same animals and therefore illustrate very accurately the changes due to mastitis.

TABLE 3

Percentage Composition of the Ash or Normal and Mastitis Milk

INVESTIGATOR	K_2O	Ca O	Na_2O	Mg O	P_2O_5	Cl
			Mastitis Milk			
Hashimoto	8.98	7.44	36.54	1.74	17.38	33.63
Schrodt	10.56	16.17	24.92	2.70	24.56	24.52
Sommer	5.08	7.52	42.37	2.88	8.76	44.64
Rosell (Recalculated)	17.85	16.86	35.73	2.08	16.41	34.12
			Normal Milk			
Sommer	24.65	23.42	8.18	2.79	26.28	13.95
Rosell (Recalculated)	19.13	29.45	15.41	2.48	32.76	15.93

Udder infections unquestionably decrease the calcium, potassium and phosphorus content of milk and increase, very markedly, the sodium and chlorine. In view of the rather delicate balance between the various ions of milk necessary for maximum stability of the casein toward heat (10) (11) (12), such changes in the mineral constituents would be expected to influence the heat coagulation point markedly. One effect, apparently, is to render the mastitis milk decidedly more heat sensitive than normal milk after concentration to a solids content comparable to that of evaporated milk. The mastitis milk seems to be unbalanced in the direction of too little calcium. This is indicated by the stabilizing effect of added

calcium acetate and also by the tendency of high forewarming temperatures to destabilize the milk more than low temperatures.

Milk from mastitis udders is low in hydrogen-ion concentration. This in itself may be an important factor in the poor stability of such milk, since Benton and Albery (11) have shown that the pH effect may overshadow the effect of added ions outside the pH ranges of 6.58 to 6.65. It may be argued therefore that the beneficial effects of adding calcium acetate to mastitis milk are a result of a lowering of the pH rather than any direct effect of the cation. It is impossible from the present data to determine which of these probabilities is the important one.

The considerable increase in the sodium chloride content of mastitis milk would increase the osmotic pressure were it not for the fact that a corresponding decrease in lactose occurs, which maintains the milk isotonic with blood (14). Nevertheless the mastitis milk contains a higher concentration of ionized electrolytes as indicated by the increased conductivity (14). The presence of this excess of electrically active particles might be expected to destabilize milk casein toward heat or any other agency of coagulation.

Another explanation of the reduced heat stability of mastitis milk might also be offered, based on the increase in the albumin content characteristic of such milk. Sommer (15) has shown experimentally that increasing the albumin content of milk lowers its resistance to coagulation in the condensed form. Since the increase in albumin content of mastitis milk, however, takes place at the apparent expense of casein (16), it seems unlikely that the increase in albumin would more than compensate the decrease in casein.

The literature dealing with the heat stability of milk lacks any definite statements regarding the presence or absence of milk from cows affected with mastitis. Apparently this factor has been neglected in most, if not all, of the studies made on the subject. It is believed that the peculiarities exhibited by some of the samples of milk used by the various workers might have been related to the presence of mastitis milk. Several of the investigators (10) (11) (12) (13) have indicated that there are two general types of milk (omitting the effects of bacterial acidity and enzymes). The most common type is that which is stabilized by high forewarming temperatures and additions of such anions as di phosphate, carbonate, citrate, borate, etc. The less common type, limited for the most part to small lots of milk or milk from individual cows, is that stabilized by additions of acid and such cations as calcium or magnesium. This latter type of milk is suspiciously similar in stability characteristics to the mastitis milk utilized in this study. Because of the wide-spread occurrence of mastitis of the sub-clinical type, it is reasonable to suppose that some of the work that has been carried out on the subject of heat coagulation has been complicated by this apparently disregarded factor.

CONCLUSIONS

There seems to be no appreciable difference between concentrated soft-curd milk and concentrated hard-curd milk with respect to senstivity toward heat coagulation and with respect to the stabilizing or destabilizing effects of forewarming and the addition of certain anions and cations.

Concentrated milk from animals infected in one or more quarters with sub-clinical mastitis is decidedly less stable toward heat than normal milk. Such milk seems to react oppositely to most normal milk in that it is further destabilized at the higher forewarming temperatures and by additions of sodium carbonate and is further stabilized by additions of calcium acetate. In these particulars, at least, it is very similar to one of the two "types" of milk noted by previous investigators, namely, that type, only occasionally encountered, which is stabilized by bacterial action, mixing with it "poor quality milk," and by additions of acids, and cations such as calcium and magnesium.

Mixing mastitis milk with normal milk tends to destabilize the latter.

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FIVE YEARS RESULTS ON MONTHLY CLIPPING OF PASTURES

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Eleven or more caged pasture areas at Baton Rouge have been clipped at monthly intervals for the growing seasons of 1930–34. Four of these areas were in seeded bench land pasture, while the others were in recently broken alluvial land. The soil is nearly neutral in reaction and high in fertility. The prevailing vegetation in early spring was White Dutch clover (*Trifolium repens*) except on bench land, where much hop clover (principally *Trifolium procumbens*) occurred. Rye grass (*Lolium* sp.), Canary grass (*Phalaris* sp.), and common oats were present in varying amounts in different seasons. This early vegetation was gradually replaced in May by Dallis (*Paspalum dilatatum*) and Bermuda (*Cynodon dactylon*) grass, with a small amount of carpet grass (*Axonopus compressus*) during wet summer months. White clover reappeared during September and October with favorable growing conditions. Some minor grasses and edible weeds occurred at times. The term "grass" is used hereafter for convenience to include all of the above edible vegetation. Inedible vegetation so far as discernible was plucked out of each area just before cutting.

Eight of the areas used were 3×7 feet and the rest 2×2 feet in size. With the exception of two areas in oats, or cage destruction by cattle, the same location was used continuously for five years. These caged areas were closely cut with a grass hook the first week in March of each year, and at approximately 30-day intervals thereafter during the growing season. The intervals were sometimes extended to avoid clipping grass on a rainy day or holiday. Because of slow growth, June and early July were combined in one cutting. The entire weight of fresh grass was recorded and individual samples taken for analysis. The average height of grass was determined by three measurements prior to clipping. It was observed during the first two years that samples of the same kind of grass cut on the same date checked closely, and thereafter one composite sample was used for similar grass each date. Analysis were by standard methods adopted by the Association of Official Agricultural Chemists.* A 400 gram portion of each sample of grass was dried to constant weight at room temperature and from this the hay yield determined. The nutritive value (T. D. N.) was calculated from the coefficient of digestibility of peas and oats for early cuttings and for those of Bermuda for grass cut after May.

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TABLE 1

Composition of pasture grasses cut monthly 1930–1934, Baton Rouge, La.

DATE OF GROWTH	RAIN FALL INCHES	NO. SAMPLES	HEIGHT INCHES	GRASS PER ACRE POUNDS	DRY MATTER PER ACRE LBS.	DRY MATTER IN 100 POUNDS GRASS (LBS.)	PER CENT OF DRY MATTER					T. D. N. PER 100 GRASS (EST.) LBS.
							Crude protein	Fat	N. F. E.	Fiber	Ash	
February	5.79	47	4.1	4,302	909	21.13	21.34	4.06	42.35	21.89	10.36	13.31
March	4.00	57	5.8	6,149	1,117	18.17	20.32	3.71	44.03	21.94	10.00	11.72
April	5.02	60	7.2	8,078	1,627	20.14	18.81	3.56	42.05	25.18	10.40	12.65
May	5.38	56	4.6	4,446	1,101	24.76	16.95	3.38	41.69	26.84	11.14	15.17
June } July }	4.27	58	5.9	4,637	1,370	29.54	13.49	2.66	46.47	27.63	9.75	14.97
August	5.96	61	8.2	7,248	1,735	23.94	12.56	3.11	44.26	29.89	10.18	12.02
September	5.12	57	7.1	5,608	1,404	25.03	11.64	3.32	45.02	29.96	10.06	12.36
October	5.35	55	4.9	3,539	1,015	28.67	12.43	3.14	44.58	29.27	10.58	14.05
Season												
1930	34.52	68		39,566	8,586	21.70	15.21	3.03	42.94	27.12	11.70	13.20
1931	32.10	95	39.5	49,979	12,330	24.67	16.81	3.45	44.97	25.31	9.46	13.38
1932	48.03	96	43.9	39,660	10,050	25.34	15.60	3.35	44.76	26.58	9.95	13.93
1933	40.00	104	52.3	41,143	9,570	23.26	15.19	2.81	44.97	27.40	9.63	12.41
1934	49.48	88	51.7	35,967	7,963	22.14	14.73	3.39	42.67	28.78	10.43	13.09
Ave. 5 yrs.	39.82	451	46.8	41,263	9,667	23.43	15.51	3.21	44.06	27.03	10.19	13.20

Table 1 presents seasonal and yearly weighted averages of these grass clippings. The date of growth is for the larger part of the month listed. "February grass" was actually cut the first week in March. Likewise, the rainfall is for the period prior to clipping, rather than for the calendar month. Because of a late season and delay in placing cages, no samples were obtained early in 1930. We have been impressed all through this work by three important points, two of which are evident in glancing at this table. First, that the grass yields reached two peaks, one for April and the other in August. The first is explained by the heavy yield of clover under the first favorable growing conditions of spring. This growth in turn so shades Bermuda and other summer grasses that several weeks of slow growth occur after the clover disappears. Distribution of rainfall is also poor as compared to late summer. Cooler night temperatures and lack of root reserve slows growth of grass in September and October. In grazing practice, one or more fields are allowed to grow for hay in May, all grazed in June and early July, and again surplus pastures cut for hay in August or September. That necessitates two or more fenced pastures for one dairy herd. Second, the early spring grass contains nearly twice as much crude protein as that cut in September. A gradual decline in protein and moisture content occurs with advancing season while fiber content increases. Fat content apparently decreases slightly with advance in season, but nitrogen free extract and total ash were quite constant. Because of these changes, the computed total digestible nutrients per 100 pounds of grass tend to rise to 15 in mid-summer and then gradually decline. If the following coefficients of digestibility suggested by the pasture committee (1) (crude protein 75; crude fiber 79; nitrogen free extract 80; and fat 50) are used for all samples, the nutritive value of all clippings average about 27 per cent higher. The difference is even greater for late summer. One hundred pounds of September grass would contain 18.06 pounds, or 46.2 per cent more total digestible nutrients by the use of these coefficients than computed in Table 1. The true value is probably somewhat less than these latter figures. As pointed out in a previous article (2), changes in protein are not as abrupt as the results indicate. The high percentage of non-protein nitrogen in immature grass gives a higher crude protein figure than actually exists. Late summer clippings are undoubtedly more mature, throughout representing the same length of growing time. Feeding practices may be advantageously changed to fit pasture yields and composition, however. A third fact brought out has been the uniformity in analysis of grass samples clipped the same date, regardless of species, genera, or even family. Italian rye grass and oats cut in early spring are very similar in chemical analysis to clovers grown during the same period. Pure samples of Dallis, Bermuda, or carpet grass cut on the same date were much alike

in analysis. Rate of growth as affected by distribution of rainfall or temperature evidently is more important than species of plant in the resulting effect on chemical composition.

Total rainfall for the period or during preceding period had a rather indirect effect on grass growth. The least rainfall occurred in 1931, the year of greatest growth, while the heaviest rainfall occurred in 1934, the year of lowest yield. Even distribution of rainfall, especially during the growing months of May, June, July, was responsible for large yields in 1931, and the poor distribution for lower yields in 1930 and 1934. In general, low average minimum and maximum temperatures within a period were recorded when the best yields were obtained. This was especially true during the spring months of clover growth.

During the first two years, nitrate and superphosphate were applied to duplicate areas. Neither had any effect on protein content of the grass. This checks with results obtained by the Florida (3), South Carolina (4), Delaware (5) stations, and other Louisiana work (6). Fertility causes faster and more total growth rather than affecting the plant's chemical composition.

Sixty composite samples, collected during the first four years and air-dried, averaged 0.989 per cent calcium oxide and 0.778 per cent phosphorus pentoxide as previously reported (7). Late summer clippings averaged much lower in calcium and slightly lower in phosphorus than spring growth. No samples were low enough to indicate that an animal might suffer from a lack of these minerals if receiving all necessary nutrients from the grass.

One observation for which we have as yet no explanation is that where cages were not moved in five years, the amount of clover appears to be less and grasses have increased in the early spring.

SUMMARY

1. Pasture yields were higher in April and August than other months when clipped at 30-day intervals for five years.

2. February grass, calculated on a dry matter basis, contained nearly twice as much crude protein as that cut in September. Calcium, and to some extent phosphorus, decreased from spring to late summer. Dry matter and fiber content increased as the season advanced, while other constituents were quite uniform.

3. Samples of grass representing the same growth period were quite similar in chemical analyses regardless of species or genera or fertilizer.

4. Distribution of rainfall, maximum and minimum temperatures, and shading are some other factors that influence rate of growth and consequent analysis and yield of pasture vegetation under the condition of this experiment.

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OXIDIZED FLAVOR IN MILK

I. THE PROBABLE RELATION OF LECITHIN TO OXIDIZED FLAVOR*

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It is generally believed that oxidized flavor is the taste-result of the oxidation of butterfat in milk. This belief has arisen from the fact that the flavor, once developed, tends to remain with the butterfat portion of milk during processing; and that the flavor never has been known to develop in skimmilk. However, one exception has been observed in the case of buttermilk, in which oxidized flavor has been known to occur (5).

In 1916 Guthrie (2) reported that his results "seemed to indicate that metallic flavor developed more rapidly and to a greater extend in cream that was rich in butterfat than in cream with a low fat content. The flavor was very strong in samples of soft-cream cheese, but was never noticed in cottage cheese which contains practically no butterfat. Whenever the flavor was found in whole milk it was always near the surface, in the cream, and it was never observed in skimmed milk. For some reason, however, it was often found in buttermilk. With the exception of buttermilk, metallic flavor was never found in a dairy product that was low in butterfat content." It is questionable whether the metallic flavor referred to by Guthrie is identical with so-called oxidized flavor. The term oxidized flavor, referring to the off-flavor caused in milk by dissolved copper or iron, sometimes has been called cappy, cardboard, metallic, oily, tallowy, oily-tallowy, emery, and corundum. Tracy, Ramsey, and Ruehe (6) have shown that small amounts of copper dissolved in milk fail to produce tallowy (oxidized) flavor when considerable growth of microorganisms has occurred, yet the metallic flavor observed by Guthrie developed in cream and buttermilk during and after the growth of lactic acid bacteria in amounts sufficient to cause souring. Nevertheless Guthrie's observations of the development of metallic flavor in sour buttermilk are strikingly similar to those of the authors in the case of the development of oxidized flavor in sweet-cream buttermilk.

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The development of oxidized flavor in buttermilk, when no other dairy product of like butterfat content supports this flavor, suggests that the lecithin adsorbed on the fat globules is the constituent first affected when oxidation occurs, and that the butterfat may be oxidized little or not at all in the average case. The finding (5) that lecithin purified from dry buttermilk and having become oxidized during purification imparts a typical oxidized flavor to skimmilk when dispersed in it lends further support to this suggestion. In addition, the report of Henderson and Roadhouse (3) that milk fat from milk of oxidized flavor has passed only a fraction of its oxygen-uptake induction period indicates little likelihood that the oxidation of the milk fat had proceeded far enough to become oxidized or to impart oxidized flavor. The facts that at least one double bond occurs in the milk-lecithin molecule; that lecithin in purified state oxidizes readily; and that lecithin makes up a portion of the adsorbed layer on the butterfat globules and, therefore, probably is exposed to the action of oxidizing agents before the butterfat, are further evidence in support of a belief that lecithin, rather than butterfat, is the constituent affected when oxidized flavor develops.

EXPERIMENTAL

Removal of Adsorbed Substances

If the constituent in which oxidized flavor develops is one of those adsorbed on the butterfat globules in milk it should be possible to obtain butterfat free of oxidized flavor by removal from the globules of adsorbed materials. Accordingly, six trials designed to accomplish this objective were planned. Five of these trials were carried out in the manner described below.

Twenty gallons of selected milk, in well-tinned ten-gallon cans, were placed in cold storage immediately after milking. Morning's milk was used in each case, and pasteurization followed milking within two hours. Pasteurization was accomplished by placing the cans of milk in a tank fitted with a water inlet and a steam jet, heating to 144° F. and holding for 30 minutes. Into one can of this milk was placed 0.01 per cent of powdered copper before pasteurization, whereas the other milk was not treated but was used as a control batch. After pasteurization both batches were cooled. The milk to which no copper had been added was cooled to about 70° F. and processed immediately by a procedure which will be described later. The milk to which powdered copper had been added was cooled to 40° F. and placed in two large glass bottles which then were immersed in ice water, and air was bubbled through the milk for 24 hours. This treatment was found to cause the development of a strong oxidized flavor. Previous experiments had shown that no off-flavors were produced by air treatment of milk to which no copper had been added. The milk

then was heated to 65 or 70° F. and separated. The cream was standardized to 35 per cent butterfat with skimmilk just separated from it.

The cream from each batch was divided into two lots. Lot 1 cream was churned in a glass churn and its butter and buttermilk tasted to detect the presence of any oxidized flavor.

Lot 2 cream was converted into washed butterfat in order to remove from the butterfat the substances adsorbed on the fat globules. The cream was diluted to a volume of about three gallons with water at approximately 100° F., separated by means of a De Laval No. 17 separator, rediluted, and separated until the cream had been washed 14 times or more. Near the end of this procedure a noticeable oiling off of the butterfat occured, which indicated that the fat globules no longer were stabilized. Further washing caused a complete oiling off of the butterfat.

In order to determine whether the treatment described above caused the removal of the lecithin contained in the film originally adsorbed on the fat globules, three different butterfats prepared in this way were analyzed for organic phosphorus. For this determination the fats were fused in a mixture of two parts sodium carbonate and one part potassium nitrate. After fusion the mixture was dissolved in hot water and hydrochloric acid and phosphorus was determined by the colorimetric method of Fiske and Subbarow (1). No trace of phosphorus was found in any of the samples, thus indicating that the washing removed all or practically all of the phospholipids adsorbed on the fat globules in the original milk.

The washed butterfat was redispersed in fresh skimmilk of good flavor by homogenizing at a pressure such that the fat globules were approximately of the same size as those in the original whole milk as shown by comparison under the microscope. Milk prepared in this way will be referred to as remade milk.

An attempt was made to develop oxidized flavor in the remade milks in order to determine whether or not development is possible in milk containing butterfat globules from which the "hull" substance has been removed. Accordingly such remade milk was treated with 0.01 per cent of copper powder, pasteurized and air bubbled through as described above except that the air treatment was continued for 36 hours.

To determine if the butter oils from these batches were oxidized, a Kreis test was made on each fat. The results did not indicate even the slightest degree of oxidation in any of the samples of butter oil.

The control batch of milk differed from the experimental batch only in that no copper was added to it and no air treatment given. It was separated fractioned in the same manner as the experimental batch.

A sixth experiment was conducted in the same manner as the five described above except that the oxidized flavor was developed by the method of Tracey, Ramsey and Ruehe (6). Their method consisted of adding a

soluble copper salt to the milk at the rate of 2.6 parts per million of copper and storing at a low temperature until the oxidized flavor developed. A storage period of 24 hours was required.

The results of the six experiments described above are shown in Table 1.

TABLE 1

The distribution of oxidized flavor in milk fractions—(Results of six trials)

FRACTION	*CONTROL	*TREATED WITH 0.01% CU
Original milk	–	–
After pasteurization	–	–
After air treatment	–	+++
Cream	–	++++
Skimmilk	–	+++
Butter	–	++++
Buttermilk	–	++++
Washed butterfat	–	+
Remade milk	–	–
Studies of Remade Milk		
1. Pasteurized with copper	–	–
2. Pasteurized without copper	–	–
No. (1) Submitted to air treatment	–	–
No. (2) Submitted to air treatment	–	–

* + Indicates oxidized flavor present.
– Indicates no oxidized flavor present.

The results of the foregoing experiments indicate that a large proportion of the oxidized flavor occurred in the substances adsorbed on the fat globules. When these substances were removed from the butterfat and the butterfat redispersed in fresh skimmilk of good flavor, no oxidized flavor could be detected in the resulting remade milk. Of still greater significance is the fact that treatment of this milk by the procedure known to cause the development of oxidized flavor in normal milk failed to develop oxidized flavor in the remade milk.

Flavor determinations on the samples of the oxidized-flavored milk showed the intensity of the flavor to be increased in cream, buttermilk, and butter, and to have about the same degree of intensity in the skimmilk as in the whole milk. An examination of the data of Holm, Wright, and Deysher (4) given in Table 2 shows that the phospholipid content of these fractions is closely correlated with the intensity of the oxidized flavor found in the fractions studied in the experiments here reported.

Use of oxidized butterfat: During the course of tasting samples of milk of oxidized flavor it seemed to the authors that the oxidized flavor

TABLE 2

** The phospholipid content of milk and its products*

PRODUCT	FAT CONTENT	PHOSPHOLIPID CONTENT
Whole milk	2.55	.152
	3.80	.158
	4.34	.153
Skimmilk	0.07	.137
	0.11	.129
	0.10	.123
Cream	27.00	.224
	34.00	.269
	42.00	.284
Butter	86.5	.224
	85.7	.247
	85.5	.269
Buttermilk	.96	.284
	1.54	.318
	1.64	.382

* Excerp.s from the data of Holm, Wright, and Deysher (16).

was not typical of that of oxidized or slightly tallowy butterfat. Accordingly an experiment was planned to make a study of this point.

Butterfat free or practically free of lecithin was prepared by the wash- and one short tube. The flask then was placed in an oven controlled at pared was placed in a liter flask, fitted with a rubber stopper through which were inserted one glass tube reaching nearly to the bottom of the flask and one short tube. The flask then was placed in an oven controlled at 75° C ± 0.5°, and oxygen was bubbled through the butter oil until it became tallowy. The tallowy butter oil then was dispersed in skimmilk of good flavor by means of homogenization, the fat percentage of the remade milk being kept at about 4 per cent.

The tallowy flavor of this remade milk was pronounced and disagreeable. By successive dilutions with normal 4 per cent milk of good flavor, milks having varying intensities of tallowy flavor were obtained. When these samples were compared with those in which oxidized flavor had been produced by means of the addition of small amounts of ferrous chloride, the flavor of the samples containing the tallowy butterfat was distinctly different from that of the samples in which oxidized flavor had been produced. Even the most highly-diluted samples containing the tallowy butterfat yielded the typical tallowy flavor of oxidized butter oil, whereas the oxidized flavor of the milk samples treated with ferrous chloride was less disagreeable and more nearly resembled what may be termed oily-

stale. The latter flavor was similar to, if not identical with, the flavor previously produced in skimmilk by the dispersion in it of oxidized lecithin.

The experiment described above was repeated and the results obtained were identical. The experiment also was repeated with the variation that the butter oil was only slightly oxidized before being dispersed in skimmilk. In this case, as before, the flavor of the remade milk was tallowy, whereas the milk in which oxidized flavor had been developed by means of the addition of ferrous chloride and storage at low temperature was distinctly different in flavor, having an oily-stale taste.

CONCLUSION

Lecithin, rather than butterfat, appears to be the constituent of milk affected when oxidized flavor develops.

These results indicate that so-called oxidized flavor is not identical with the tallowy flavor of oxidized butterfat.

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A STUDY OF FACTORS INFLUENCING THE SEPARATION OF WHEY IN ICE CREAM MIXES CONTAINING VEGETABLE STABILIZERS*

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INTRODUCTION

Various vegetable products, composed chiefly of gums, have been placed on the market as ice cream stabilizers. Because of their lower cost, these substances have been suggested for use in place of gelatine to stabilize the ice cream mix, and to aid in producing a smooth ice cream, free from objectionable ice crystals.

Studies on the efficiency of the vegetable stabilizers have been made by Prescott, Heifetz, and Stanley (1), Caulfield and Martin (2), and Lucas and Gould (3). All of these investigators noted a wheying off of the ice cream mix containing certain of the vegetable products after a few days storage. The solids of the mix showed a tendency to rise to the surface leaving a comparatively clear whey in the lower portion of the container.

Caulfield and Martin (2), assumed the wheying off of the mix to be caused by an enzyme present in the vegetable stabilizer since heating of the mix to 175° F. for 5 minutes decreased the whey separation. In another study (4), it was noted that the separation increased with an increase in the storage temperature, and decreased as the storage temperature was lowered.

SCOPE OF INVESTIGATION

Because of the lack of information regarding the separation of whey in ice cream mixes stabilized with vegetable products, and because of its direct commercial importance, this defect was subjected to a more intensive study to determine the effects of certain practices on the extent of the whey separation.

The several factors considered in this study are: variations in the amount of stabilizer used; heating the stabilizer; different temperatures of pasteurization of the mix; heating the milk products used in the ice cream mix; varying amounts of serum solids; varying amounts of fat; and incomplete cooling of the ice cream mix.

PROCEDURE

Only one stabilizer was selected for this study. The vegetable product used was the one causing the greatest wheying off of the ice cream mix in

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a previous study (3). This was also the stabilizer listed as "C" in the work of Caulfield & Martin (2) who found that the product contained 9.79 per cent moisture, 35.5 per cent gum, 50 per cent reducing sugar, 2.75 per cent protein, and 0.45 per cent ash. It was thought that the findings secured from a study of this one stabilizer could be applied as well to those other stabilizers which, when used in an ice cream mix, bring about whey separation to a greater or lesser degree.

An ice cream mix containing 12 per cent fat, 15 per cent sugar, and 10 per cent serum solids was used. The ingredients of the mix were 30 per cent cream, 4 per cent milk, dried skim milk testing 97 per cent serum solids, and the stabilizer. Unless otherwise specified, approximately 0.4 per cent of the stabilizer was used throughout the trials. The stabilizer was added with the sugar after the mix had been brought to a temperature of about 100° F.

Small eighteen pound batches were prepared, pasteurized at the desired temperature in three-gallon cans in a water bath, and then viscolized. The batches were cooled to about 75° F. by pouring over a tubular surface cooler through which water was running. Small samples of each batch were further cooled to 50° F. by placing them in ice water. After cooling, samples were placed in 100 cc. graduates and stored in an electric refrigerator maintaining a temperature between 42° F. and 46° F. The whey separation was observed after varying periods of storage.

In those trials in which the powdered stabilizer was heated, the heating was carried out by using the solids plate and solids vacuum oven of a Mojonnier tester. The solids plate was maintained at 180° C. and the vacuum oven at 100° C. A vacuum of 20 inches was maintained in the vacuum oven during the drying. The powder was spread uniformly over the bottom of a flat pan approximately five by nine inches in size.

When either a suspension of the stabilizer or a mixture of the milk products used in the mix was heated to high temperatures, the substance being heated was placed in a round bottom glass flask suspended in a salt solution.

RESULTS

The Effect on the Whey Separation of Varying Amounts of the Stabilizer

Although the recommended amount of the stabilizer to use was 0.4 per cent, variations by 0.1 per cent in the concentration from none to 0.5 per cent were tried, and the mix examined for whey separation after different periods of storage. The results are given in table 1.

The results showed that concentrations of the stabilizer as low as 0.1 per cent caused a separation of the whey. However, it was observed that in the samples containing 0.1 per cent, the separation was never as distinct

TABLE 1
The effect on whey separation when different amounts of the stabilizer were used.
(Ave. 4 trials)

PER CENT STABILIZER USED	PER CENT WHEY SEPARATION AFTER				
	4 hrs.	8 hrs.	24 hrs.	48 hrs.	72 hrs.
0.0	0.00	0.00	0.0	0.0	0.0
0.1	0.00	0.20	1.5	4.0	8.0
0.2	0.56	1.70	7.0	23.0	27.0
0.3	0.25	2.30	17.0	43.0	51.0
0.4	0.00	0.20	14.0	41.0	49.0
0.5	0.00	0.20	17.0	47.0	51.0

as in those samples with larger amounts of the stabilizer. Furthermore, the separated whey was usually extremely milky in appearance.

It is interesting to note that the whey separation appeared more rapidly in the samples containing only 0.3 to 0.4 per cent than in those containing 0.5 per cent. The appearance of whey after only four hours of storage did not occur generally throughout the trials. After eight hours of storage, however, many of the samples containing 0.3 and 0.4 per cent of the stabilizer showed slight whey separation.

After 24 hours of storage, the amount of whey separation varied directly with the amount of the stabilizer used. There was little difference between the total whey separation after 72 hours in the samples containing 0.3 per cent or more of the stabilizer. An average of about 50 per cent of whey showed at the final reading.

The Effect on Whey Separation of Different Temperatures of Pasteurization of the Mix

Since it had previously been noted (2) that heating the ice cream mix containing the vegetable stabilizer to 175° F. for about five minutes decreased the amount of whey separation, a further study was made to ascertain the effect of different pasteurizing temperatures on the appearance of whey.

The ice cream mix was divided into three lots. Lot 1 was pasteurized at 145° F. for 30 minutes, Lot 2 was pasteurized at 165° F. for 30 minutes,

TABLE 2
The effect on whey separation when the mix was pasteurized at different temperatures.
(Ave. 6 trials)

LOT NO.	TEMP. OF PAST (°F.)	PER CENT WHEY SEPARATION AFTER			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	145	4.0	23.0	38.00	45.0
2	165	0.3	5.0	17.00	26.0
3	175	0.0	0.2	1.4	4.7

and Lot 3 was pasteurized at 175° for 30 minutes. The amount of whey separation noted in these samples after storage is given in table 2.

These averages show distinctly the beneficial influence of higher pasteurizing temperatures on eliminating, to a large extent, the separation of whey. Apparently, there is an inverse relationship between the temperature of pasteurization and the whey separation. It is interesting to note, however, that the highest pasteurizing temperature of 175° F. for 30 minutes did not entirely eliminate the trouble.

The Effect on Whey Separation of Drying and Heating the Stabilizer

To determine specifically if the stabilizer alone contained the causative agent of whey separation, the stabilizer, both in powdered form and in suspension, was subjected to various conditions of desiccation and heat before using in the mix.

A study was made first on the effect of abnormal conditions of drying the powdered stabilizer on the whey separation of the mix. The drying was carried out by using the solids oven of the Mojonnier tester in the manner outlined in the procedure. The results, as shown in table 4 indicate that the whey separation is not retarded by drying the powdered stabilizer for periods as long as 60 minutes. In fact, the few trials of the longer heating periods show the heating to actually increase the whey separation, but this increase may be due to other factors involved in the processing of the mix.

TABLE 3

The effect on whey separation when the powdered stabilizer was dried at 100° C. in 20 inches of vacuum for varying lengths of time

SAMPLE	NO. OF TRIALS	LENGTH OF TIME HEATED	PER CENT OF WHEY SEPARATION AFTER		
			24 hrs.	48 hrs.	72 hrs.
1	6	0 min.	0.8	13	30
2	3	10 "	1.5	14	27
3	4	20 "	3.0	13	26
4	3	30 "	10.0	32	47
5	3	40 "	9.0	34	49
6	2	50 "	2.0	26	46
7	2	60 "	.....	20	39

Since the drying of the powdered stabilizer at 100° C. did not influence the whey separation, it was thought that possibly more efficient means of heating the stabilizer could be obtained if the stabilizer were first put into solution and this solution heated. It appeared logical to assume that if the agent causing the whey separation was present in the stabilizer and was enzymic in nature, it could be inactivated by heating to near boiling

temperature. A method of heating the stabilizer in solution, therefore, appeared to be the most satisfactory.

A water solution of the stabilizer was prepared which contained enough stabilizer to make a concentration of 0.4 per cent in the ice cream mix. The solution was heated to 200° F. to 205° F. for two and one-half hours. It was then cooled and added to the ice cream mix. The mix was processed in a normal fashion and the whey separation observed after varying intervals. The results obtained are shown in table 4.

TABLE 4

The effect on whey separation when a solution of the stabilizer was heated to a high temperature before adding to the mix

TRIAL	PER CENT OF WHEY SEPARATION AFTER			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	1.0	2	9	18
2	0.0	5	12	22
3	1.5	9	19	32
4	0.0	0	1	3
5	0.0	0	1	3
6	0.5	5	13	21
7	2.0	3	6	11
8	.....	5	18	27
9	.....	5	18	28
Ave.	0.7	3.8	10.8	18.3

When these results are compared with those in table 3, it may be noted that the heating of the stabilizer in solution retarded the whey separation to some extent. However, the separation of whey was by no means eliminated even with this high heat treatment.

The Effect on Whey Separation of Heating the Milk Products Used in the Ice Cream Mix

Another possibility of a cause for at least part of the whey separation of the mix is the milk and milk products it contains. In order to determine if some property of the milk, cream, or dried skimmilk was aiding or completely causing the "wheying off" of the mix, another pasteurizing procedure was followed.

The mix was prepared as before but without the stabilizer and sugar. It was divided into three lots which were pasteurized for 30 minutes at 145° F., 165° F., and 175° F., respectively. After pasteurizing, the mix was cooled to 145° F. where the stabilizer and sugar were stirred into the respective lots. The mixes were then processed in the usual manner. The per cent of separation of whey as noted after varying storage periods is given in table 5.

TABLE 5

The effect on whey separation when the milk products were pasteurized at different temperatures before adding the stabilizer. (Ave. 8 trials)

LOT	PAST. TEMP. (°F.)	PER CENT OF WHEY SEPARATION AFTER			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	145	7.0	26	37	41
2	165	0.3	4	13	22
3	175	0.0	1	5	10

The results of these eight trials show the higher pasteurizing temperatures to bring about a decided decrease in both the rapidity of appearance of whey separation, and in the total amount of whey noted after 96 hours. When the milk products were pasteurized to 175° F. for 30 minutes no whey separation was observed in the mix for 48 hours, and then the amount was small. In the lots pasteurized at 145° F. and 165° F. whey separation was observed usually at 24 hours although the amount appearing in the 165° F. lot was usually very small, or, in some trials, no separation was detected.

To determine if the wheying off of the mix could be entirely prevented by heating the milk products, the milk, cream, and skimmilk powder were heated to 205° F. for one hour. They were then cooled to 145° F. and the mixture of sugar and stabilizer added. The mix was further processed as previous mixes. The results are given in table 6.

TABLE 6

The effect on whey separation when the milk products of the mix were heated to 205° F. for one hour

TRIALS	PER CENT WHEY SEPARATION AFTER			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
1	0.0	0.0	0.00	0.25
2	0.0	0.0	0.00	0.00
3	0.0	0.0	0.00	0.10
4	0.0	0.5	1.00	2.50
5	0.0	0.0	0.00	0.75
6	0.0	0.0	0.25	0.75
Ave.	0.0	0.08	0.2	0.72

These results show that the heating of the milk products to an extremely high temperature greatly reduced the tendency of the mix to show whey separation. In only one of the six trials was any whey observed after 48 hours of storage, and in only two trials was any observed after 72 hours of storage. In the majority of the trials a slight amount of whey separation was noted after 86 hours of storage, but in only one (Trial 4) did the separation exceed one per cent.

The Effect on Whey Separation of Different Percentages of Serum Solids in the Mix

The composition of the mix was varied so that the serum solids content of three lots was respectively 8, 10, and 12 per cent serum solids. The whey separation observed after various storage periods is recorded in table 7.

TABLE 7

The effect on whey separation when different percentages of serum solids were used in the mix. (Ave. 4 trials)

PER CENT SERUM SOLIDS	PER CENT WHEY SEPARATION AFTER			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
8	5.0	15	20	25
10	8.0	19	25	31
12	5.0	11	19	30

These results indicate that there is no correlation between the percentage of serum solids of the mix and the amount of whey separation.

The Effect on Whey Separation of Different Percentages of Fat in the Mix

Maintaining the serum solids content of the mix at 10 per cent, mixes were prepared containing respectively 10, 12, and 14 per cent of fat. The results are given in table 8.

TABLE 8

The effects on whey separation when different percentages of fat were used in the mix. (Ave. 8 trials)

PER CENT FAT	PER CENT WHEY SEPARATION AFTER			
	24 hrs.	48 hrs.	72 hrs.	96 hrs.
10	42	48	52	54
12	18	22	24	27
14	17	24	26	30

These figures show that the fat may have an influence on the amount of whey separation. A comparatively low fat content of the mix, *i.e.*, 10 per cent, seemed to favor greater whey separation than did a higher percentage of 12 to 14 per cent. However, this did not hold strictly true in all the trials. There was no noticeable difference in the amount of whey appearing in the higher fat mixes.

The Effect on Whey Separation of Incomplete Cooling of the Mix After Pasteurization

After pasteurization and viscolization, the mix was divided into two lots. Lot I was cooled by ice water to 50° F., while Lot II was cooled only

to 65° F. They were then stored and examined at proper intervals for whey separation. The results are given in table 9.

TABLE 9

The effect on whey separation of incomplete cooling of the mix. (Ave. 6 trials)

LOT NO.	FINAL TEMP. BEFORE STORING (°F.)	PER CENT WHEY SEPARATION AFTER			
		24 hrs.	48 hrs.	72 hrs.	96 hrs.
I	50	11	25	44	48
II	65	13	27	45	48

The values shown in this table indicate that the incomplete cooling of the mix had no effect on the whey separation. Undoubtedly, the temperature of the storage room is an important factor in this connection as previously shown (4).

SUMMARY

1. Whey separation was observed in samples containing as little as 0.1 per cent of the vegetable stabilizer after 96 hours of storage at 42°–46° F.

2. Using normal amounts of the stabilizer, *i.e.*, from 0.3 per cent to 0.5 per cent, and under the conditions of the experiment, a whey separation of approximately 50 per cent was noted after four days' storage.

3. Pasteurization of the mix was carried out at temperatures of 145° F., 165° F. and 175° F. for 30 minutes. The use of higher temperatures for pasteurization caused less whey separation. After 96 hours of storage the samples pasteurized at 145° F. showed 26 per cent separation, and those pasteurized at 175° F. showed an average of 4.7 per cent whey separation. The higher temperatures also retarded the rapidity with which the whey separation appeared.

4. Drying of the powdered stabilizer at 100° C. and under 20 inches of vacuum for as long as one hour did not affect its property of causing whey separation.

5. Heating a water suspension of the stabilizer to 205° F. for two hours retarded the rate and diminished the total amount of whey separation appearing in the mix when the stabilizer was used. It was, however, not reduced to the extent expected. An average of about 18 per cent of whey was observed after 96 hours of storage.

6. Pasteurization of the milk products to 165° F. or 175° F. for 30 minutes before adding the stabilizer, brought about a reduction in the whey separation which was not greatly different from the results obtained by pasteurization of the products plus the stabilizer at similar temperatures.

7. The greatest reduction in whey separation was obtained when the milk products used in the mix, *i.e.*, milk, cream, and skimmilk powder, were heated to 205° for one hour before the stabilizer was stirred in at 145° F.

By this method the separation of whey was negligible after 72 hours of storage, and showed an average of less than one per cent after 96 hours.

8. The percentage of serum solids in the ice cream mix had no effect on the whey separation.

9. A mix having a low percentage of fat seemed to favor the separation of whey more than a 12 or 14 per cent fat mix.

10. Incomplete cooling of the mix had no influence on the whey separation provided the mix was cooled to about 65° F. after pasteurization.

CONCLUSIONS

From the results obtained in this study, it would seem that the principal factor involved in the whey separation is concerned chiefly with the milk products used in the mix, rather than with the stabilizer. There apparently is a reaction, or combination, between the stabilizer used and some natural constituent of the milk, cream, and skimmilk powder to bring about the separation of whey.

This natural constituent of the milk products is, at least partly, heat labile. The whey separation may be due to enzymic action, but this does not appear to be so logical when it is considered that heating of the milk products for an hour did not entirely eliminate the defect. The proteins are probably closely involved with the separation as well as certain salts of the mix.

The elimination of the separation of whey by any practical plant method does not appear feasible. When a stabilizer is used which causes whey separation, the use of high temperatures of pasteurization is perhaps the best means of limiting the extent of the separation.

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THE NEUTRALIZING POWER OF FORAGE CROPS FOR ORGANIC AND MINERAL ACIDS

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Plant materials differ widely in their chemical composition. In most cases cereals and grasses contain a smaller percentage of basic materials such as calcium, potassium, magnesium and nitrogen and a larger percentage of readily fermentable carbohydrates than do the legumes. These differences in the ratio of the fermentable sugars to the basic constituents in the non-legumes and in the legumes may have a definite effect on the quality of the silage that can be made from these forage crops.

SUGGESTIONS FROM PREVIOUS WORK

The comparative neutralizing power of non-leguminous and leguminous types of silage materials for organic and mineral acids is unknown. Lemmerman (3) made extracts of Graminae and Leguminosae by boiling the plant roots in water and using a hand press. He observed that three to four times as much barium hydrate was required to neutralize the acids in the extracts of vetch and white clover as was required to neutralize the acids in the extracts of timothy and buckwheat. This shows a fundamental difference between these two types of plant materials.

It was pointed out by Wright and Shaw (7) that both reducing and non-reducing sugars disappear almost completely during the fermentation of both corn and soybean silages and that these sugars are converted into organic acids which preserve the material. Blish (2) concluded that the kind of acids produced was more important than the amount of acids. His data show that the quality of two samples of sunflower silage was good despite the fact that the amount of acids of one was less than one-half that of the other and that a third sample which contained an amount of acids between that of these two completely spoiled. This spoiled sample contained a strong odor of butyric acid while the others did not. The chemical analysis shows that the sugar content of the plant materials which produced the best type of acidity and thus a good silage was seven times that of the sugar crop that completely spoiled. The protein content of these samples was about the same. This may indicate that a wide ratio between the fermentable carbohydrates and the other materials such as nitrogen must be present before good silage can be expected. In fact Blackshaw (1) acted on this suggestion and studied the ratio between the protein and the fermentable carbohydrates of the more common crops grown and used for silage making in Rhodesia and their ability to produce good silage. He

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found that maize which is relied upon to produce good silage surpassed in sugar content all the other crops examined.

Wright and Shaw (6, 7) concluded that the content of fermentable carbohydrates is sufficient to produce the proper degree of acidity in the silage from any green crop, barring a few exceptional cases, and that the only other chemical qualification necessary to a succulent silage is the proper moisture content. They state that any forage crop which is palatable as a forage crop will be palatable as silage if the moisture content is right.

A WORKING VIEWPOINT

Some important determinations and points in silage making which have been made and observed by previous investigators suggest a new working viewpoint. It is presented briefly. The successful natural conversion of plant materials into silage is accompanied by the formation of organic acids. As the acidity of the fermenting plant materials approaches a certain pH value the fermentation is automatically retarded. If an insufficient quantity of acid producing carbohydrates is available and the proper degree of acidity fails to develop either from this cause or for any reason such as the neutralizing effect of the plant bases on the acids, putrefaction of the ensiling material may occur to a larger or smaller degree and the quality of the product may be materially reduced accordingly. This suggests that in such a process cereals, grasses and other non-leguminous plant materials which have been used successfully in making silage contain more sugar and less basic substances like calcium and nitrogen than the legumes such as clover, alfalfa and soybeans which usually produce poor silage or putrify, and that these basic substances in the leguminous materials neutralize and adsorb to a larger extent the acids which are produced in fermentation as fast as they are formed than do the non-leguminous materials. This may indicate that a larger quantity of acids must be produced in leguminous silage than in non-leguminous silage if the degree of acidity of the two materials is to be the same at the end of the fermentation processes. Also, if the first acids produced in a legume silage fermentation are used up in combining with bases it allows considerable time for a certain amount of putrefaction to occur before enough acidity develops to interfere with the putrefactive process. These fundamental differences in the character of the two types of plant materials may result not only in different types of fermentation but also in different qualities of the fermented product.

The acids produced in such fermentations which seem most desirable are lactic, acetic and propionic. The quantity of each acid formed is probably related to the environmental conditions, such as temperature and moisture. Only a certain amount of these acids is ever produced. Apparently their formation is controlled in the silo, where anaerobic conditions largely prevail, by the supply of sugars that the plant materials contain.

PROBLEM AND METHODS

With the above viewpoint in evidence a study of the neutralizing power of leguminous and non-leguminous forage crops as they relate to the production of silage seemed desirable. Data obtained from such a study are presented in this paper. It is believed that such data may lead to a better understanding of the practices necessary to properly ensile leguminous forage crops. This has a special bearing in New York State where poor weather conditions often prevent the process of hay making from freshly cut material.

Since silage can be made readily from non-legumes and with considerable difficulty from legumes it would seem that a study of this problem in relation to the basic substances that the plant materials contain as measured by their power to neutralize acids may aid in understanding the frequent spoilage of silage due to undesirable fermentations.

A wide variety of crops and plant materials were used in this study in order to obtain a true picture of the neutralizing and adsorbing power of silage materials. They represent both legume and non-legume forage crops. They were obtained at a stage of growth at which they normally would be taken for hay, fodder, silage or seed. After drying they were ground in a Wiley mill so that acids could rapidly penetrate into every particle. One gram portions of the chaffed crop materials were placed in beakers and 25 ml. distilled water added. A definite quantity of acid was then added to the suspensions. Although the action of the crop materials on the acids seemed to be immediate, the pH reading of the mixtures was not recorded until after the suspensions had stood for at least five minutes. Each of the acids was balanced so that 30 ml. would exactly neutralize 30 ml. of a 0.1 normal NaOH solution. This provided equal quantities of acid in each solution. The degree of acidity of the crop materials before and after the addition of the acids was determined by the quinhydrone method. It was assumed that the pH readings thus obtained would indicate the neutralizing and adsorbing capacity of the chaffed one gram portions. It was desired to express the intensity factor of acidity rather than the quantity of acid substances present because it is known that only a few organisms can produce from soluble carbohydrates an intensity factor greater than that expressed by pH 4.5 and because this seems to be near the intensity required to successfully preserve silage materials. It was desired therefore to know how much acid was required to produce an intensity factor near this figure. At the Ontario Agricultural College (4) the colorimetric method was used for pH determinations. The lowest intensity factor of acidity found at any time during the fermentation of sweet clover was pH 4.4 although the freshly cut samples ranged from pH 6.6 to 6.9. This report from the Ontario Agricultural College states that good corn

silages which were tested by this method gave an average intensity factor of pH 4.0.

RESULTS

The intensity factor of a water-suspension of one gram of each non-leguminous plant material before and after being exposed to 3 ml. of 0.1 normal acids is given in table 1.

TABLE 1

Neutralizing power of forage crops for organic and mineral acids

3 ml N 0.1 acid	Non-leguminous crop material and pH					
	Corn	Sorghum	Foxtail Grass	Barley Hay	Blue Grass 274	Blue Grass 275
Check	5.8	5.7	6.1	6.5	5.6	5.6
Acetic	4.4	4.3	4.5	4.8	4.6	4.6
Citric	4.3	4.2	4.5	4.7	4.4	.....
Phosphoric	4.2	4.0	4.4	4.7	4.5	4.4
Lactic	3.9	3.8	4.1	4.4	4.0	4.1
Hydrochloric	3.4	3.4	3.8	4.1	3.7	3.6
Sulphuric	3.3*	3.3*	3.7	3.9	3.6	3.6
5 ml N 0.1 acid	**Leguminous crop material and pH**					
	Vetch	Alfalfa	Alsike Clover	Pea Vines	Pea Flour	Soybean Hay
Check	6.0	5.7	5.8	5.7	6.2	5.6
Acetic	4.8	4.6	4.6	4.7	4.5	4.7
Citric	4.6	4.5	.....	.....	.....	4.4
Phosphoric	4.6	4.4	4.4	4.5	3.9	4.6
Lactic	4.2	4.1	4.1	4.3	3.7	4.4
Hydrochloric	4.0	3.8	3.8	4.1	3.3*	4.3
Sulphuric	3.9	3.7	3.7	4.0	3.3*	4.2

* Estimated from E. M. F.

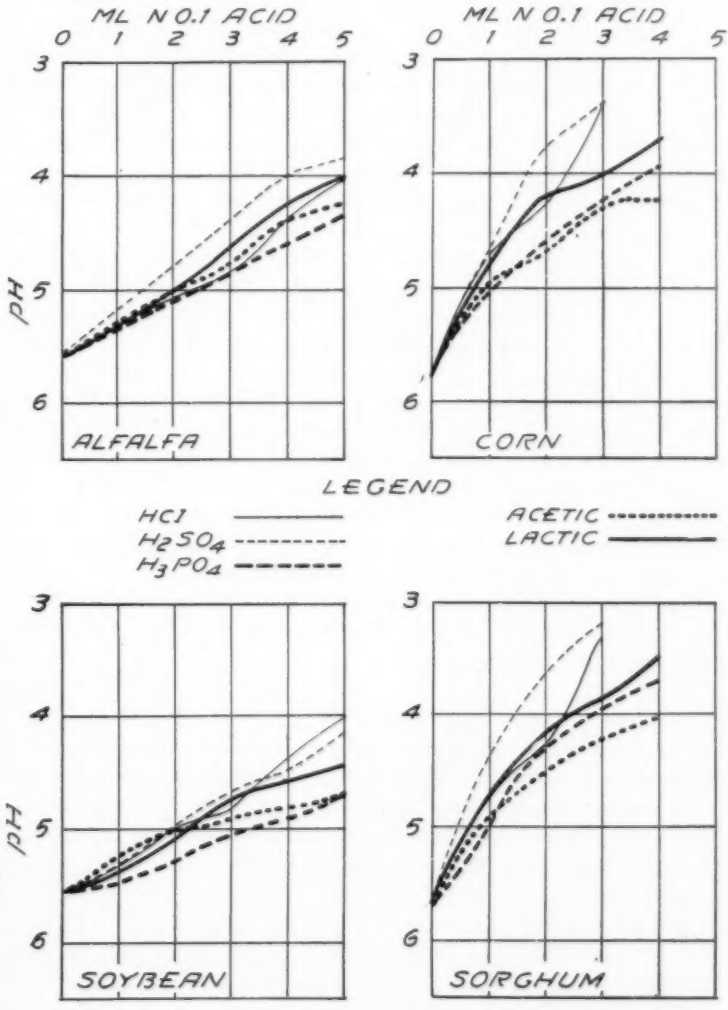
It is evident that plant materials vary considerably in their capacity to neutralize and adsorb organic and inorganic acids. One gram of chaffed corn suspended in 25 ml. of water was changed from pH 5.8 to pH 4.4 by the addition of 3 ml. of 0.1 N acetic acid and to pH 3.3 by the same quantity of 0.1 N sulphuric acid. The effect of the corn suspension on the other acids lies between that of acetic on the one hand and of sulphuric on the other, the order being acetic, citric, phosphoric, lactic, hydrochloric and sulphuric. All non-legume crop materials tested respond to acid treatment in this same order.

Various crop materials did not exhibit the same intensity factor when treated with an identical quantity of acid. A suspension of corn, the reaction of which was pH 5.8, was changed to pH 3.3 by 3 ml. of a 0.1 N H_2SO_4 while an oat straw suspension, the reaction of which was normally pH 6.5,

when treated with the same quantity of acid was changed to pH 4.1; and the oat grain of pH 6.2 to an acidity of about pH 2.5.

The intensity factor of water-suspensions of one gram of each leguminous plant material before and after being exposed to 5 ml. of 0.1 normal acid is given also in table 1.

It will be observed that the acidity of these legume crop materials as



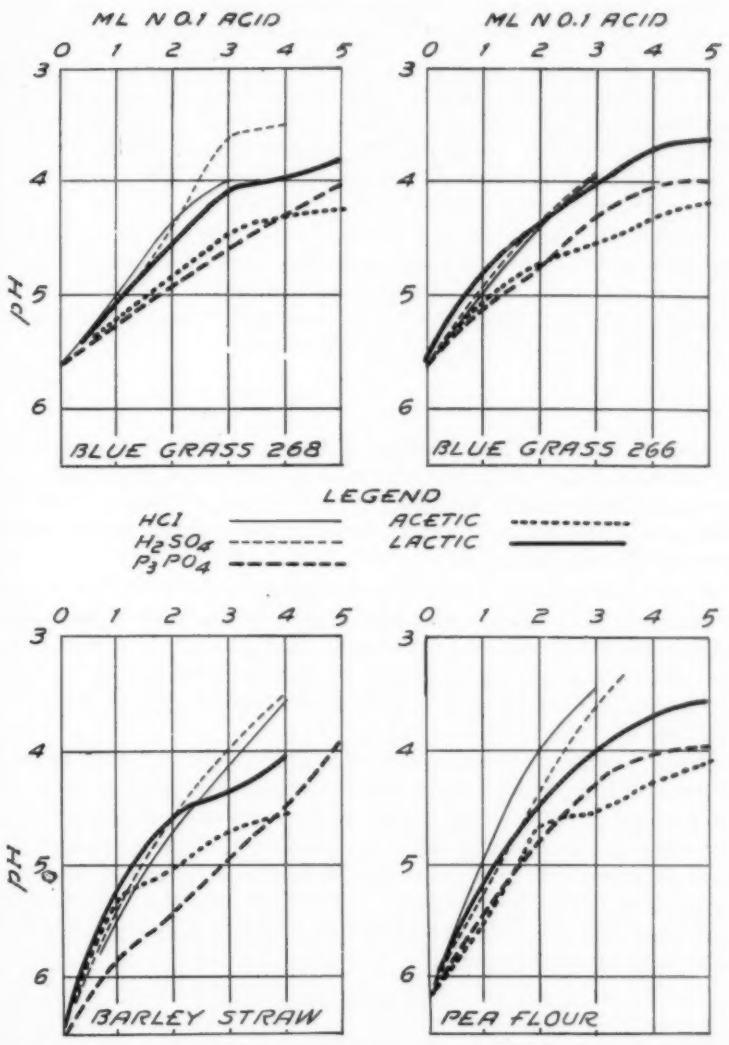
expressed by pH figures before being treated with acids was about the same as that of the non-legumes.

The slightly acid legume crop materials show considerable difference in the intensity of acidity they possess after having been exposed to different acids. A water-suspension of one gram of vetch the acidity of which was pH 6.0 was changed to pH 4.8 by the addition of 5 ml. of 0.1 N acetic acid and a similar quantity of vetch was changed to pH 3.9 by the same quantity of 0.1 N sulphuric acid. The change in acidity of these legume plant materials was increased by the six acids in about the same order as the acidity of the suspensions of the non-legumes was increased but the increase, however, was much less. From the data presented it is difficult to say that the chaffed material of the legumes adsorbed and neutralized more of the citric acid than of the phosphoric acid.

Leguminous and non-leguminous plant materials adsorbed and neutralized larger quantities of organic and mineral acids, as measured by a change in pH values, when they were exposed to larger quantities of the acids. The leguminous materials were more active in this respect than were the materials of a non-leguminous nature. This relationship is shown in graphs 1 to 8. It suggests that the plant materials both adsorb and neutralize the acids. If this were not so it would seem that if the plant materials after having been exposed to acids were then exposed to increasing increments of sodium hydroxide of a strength equal to that of the acids employed, they would neutralize the acid and give the same curve that was obtained when becoming more acid. This apparently does not occur. Chaffed material of Sorghum, the reaction of which was pH 5.7, was treated with 1 ml. increments of N 0.1 H_2SO_4 until its reaction was about pH 3.3, and a curve thus obtained. After this sodium hydroxide in the same quantities and of the same strength as that of the H_4SO_2 was added to the suspension where the pH was 3.3. The suspension did not return to the original but to a much higher pH.

DISCUSSION

The data presented in this paper indicate that leguminous crop materials both adsorb and neutralize organic and inorganic acids to a larger extent than do non-leguminous forage materials. In the normal fermentation of leguminous crop materials this is taken to indicate that considerably larger amounts of organic acids must be produced to effect a pH change of 1 unit than would be required if non-leguminous materials were being ensiled. Such a delay in effecting the pH necessary to preserve this material may permit the liberation of a slight quantity of ammonia from the highly nitrogenous material. If this occurs still more acid is required. This beginning of putrefaction, accompanied by the slow decrease in pH may



persist until all of the easily fermentable carbohydrates is utilized. When this situation is reached excessive putrefaction becomes evident and the leguminous forage material that should have produced silage is spoiled and is no longer suitable for animal consumption.

The pH of plant materials whether leguminous or non-leguminous was changed the least by a given quantity of acetic acid and most by the same

quantity of sulphuric acid. This was most pronounced at a pH 4.5 to 3.8, although evident at a higher pH. The order of effectiveness seemed to be acetic, citric, phosphoric, lactic, hydrochloric and sulphuric. The fact that hydrochloric and sulphuric acids are more effective in neutralizing the basic nature of plant materials, whether leguminous or non-leguminous than acetic, citric, phosphoric or lactic is probably the reason why Virtanen (5) recommends the employment of these acids in the artificial preservation of highly nitrogenous forage crops.

Whether these acids can be profitably used to augment or supplant the normal formation of acids in silage production is an economic question that still deserves attention. The difficulties encountered in making hay of such crops as the first cutting of alfalfa together with the losses that often occur emphasize the importance of such an undertaking.

SUMMARY AND CONCLUSION

A study was made of the ability of forage crops to neutralize organic and mineral acids. Such crops as alfalfa, vetch, timothy, barley, oats, corn and bluegrass were employed. Finely ground portions of such crops were exposed to acids of known strength and any change in the intensity factor of the material noted.

It was observed that leguminous materials require more acid to bring about a change of 1 pH unit than was required by non-leguminous materials and that more acid was required by both types of material to change their reaction 1 unit in the region of pH 4.0 than was required in the region of pH 6.5. The strong mineral acids were more effective in this respect than were the organic acids.

From the data presented it seems doubtful whether certain leguminous materials contain enough fermentable carbohydrate material if it were converted quantitatively into organic acids to produce an intensity factor comparable to that found by other investigators in the good types of silage which have been produced from crop materials containing a larger percentage of fermentable sugars. It is concluded that this is probably the main reason why many failures have been recorded in attempts to produce silage from leguminous crops.

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THE DETECTION OF FORMALDEHYDE IN MILK BY MEANS OF THE METHYLENE BLUE REDUCTION TEST[1]

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That the addition of formaldehyde to milk decreases the bacterial content and prolongs the keeping quality has been known by dairymen for many years. Although suitable tests are available for the detection of formaldehyde in milk, these tests are not usually made unless the suspicion of the purchaser has been aroused.

Schardinger[1] observed that the addition of a formaldehyde-methylene blue solution to milk caused a rapid decolorization of the dye to its leucobase. From Schardinger's observations it is easy to deduce that milk to which formaldehyde has been added should likewise show a short reduction time when subjected to the routine methylene blue reduction test. Inquiry at several milk receiving stations where the methylene blue reduction test was employed confirmed this deduction and revealed that confusion had frequently arisen from analyses of individual samples of milk which showed a short reduction time (less than 30 minutes) and at the same time, a low plate count. Analyses were made for formaldehyde in several samples showing such anomalous results, and in each case the test was found to be positive for this preservative.

The wide-spread use of the methylene blue reduction test in dairy plants suggests the possibility that this test may offer a practical means of identifying the samples of milk to which formaldehyde has been added. A short reduction time certainly could not be regarded as convincing evidence that formaldehyde had been added to milk, but it might serve as a basis for more intelligent selection of the samples to which sensitive tests for formaldehyde were to be applied.

METHODS

Several preliminary experiments were performed to determine the general range of dilutions of formaldehyde which affects the bacterial count and the reduction time of milk. The methods employed and the results obtained in these preliminary experiments were essentially the same as those of the final experiment presented in this paper.

By suitable serial dilution, concentrations of formaldehyde ranging

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[1]Contribution No. 160, Department of Bacteriology.

[2] Schardinger, F. 1902. Über das Verhalten der Kuhmilch gegen Methyleneblau und seine Verwendung zur Unterscheidung von ungekochter und gekochter Milch. Zeit. Unters. Nahr. Genuss. 5: 1113–1121.

from 1:100 to 1:1000,000 were prepared. (These concentrations are calculated on a basis of actual HCHO and not as commercial formalin). Standard plate counts were made on the raw milk before it was treated with formaldehyde. Immediately after the various dilutions of formaldehyde had been prepared duplicate methylene blue reduction tests were set up. After the milk had been in contact with the formaldehyde for 24 hours at 21° C., standard plate counts and methylene blue reduction tests were again made.

RESULTS

The data in the accompanying table show the effect of various concentrations of formaldehyde on the bacterial growth, reduction time, coagulation time, and taste of the milk.

Bacterial growth. The standard plate count on the original milk was 18,000 per cc. After 24 hours at 21° C. the milk containing formaldehyde in concentrations of 1:2500 or more showed no colonies on 1 in 10 dilution plates. The numbers of bacteria were markedly decreased by concentrations of formaldehyde up to 1:20,000, slight growth was evident in the presence of 1:25,000, and appreciable growth occurred in the presence of higher dilutions of formaldehyde. As may be seen from the results in the table, the standard plate count increased in 24 hours from 18,000 to 470,000 per cc. in the presence of a 1:30,000 concentration of formaldehyde. Within the limitations imposed by observations of a single experiment, these results suggest that the addition of formaldehyde in concentrations of less than 1:25,000 failed to retard bacterial growth sufficiently to keep the count below the usual bacterial standards for market milk. Results of experiments not included in this paper indicate that the minimum concentration of formaldehyde required to inhibit growth varies with different samples of milk, and in general the higher the bacterial content of the milk the higher the concentration of formaldehyde required.

Methylene blue reduction time. The reduction time observed for the original sample of milk without formaldehyde was 660 minutes; the addition of 1:100,000 concentration of formaldehyde shortened the reduction time to 259 minutes. Similarly, increasing quantities of formaldehyde shortened the reduction time progressively to as low as 21 minutes in the case of a 1:1000 concentration. However, when a 1:250 concentration of formaldehyde was added the reduction time was 440 minutes and a 1:100 concentration inhibited reduction.

When formaldehyde was added to this milk in concentrations between 1:1000 and 1:10,000 the reduction time was decreased to a few minutes. It is apparent that the short reduction times observed in the presence of 1:1000 to 1:10,000 concentrations is largely, if not wholly, the result of chemical rather than of biological factors. When sufficient formaldehyde

The effect of formaldehyde on the bacterial count, reduction time, coagulation time and taste of milk

DILUTION OF FORMALDEHYDE 1 :	BACTERIAL COUNT		REDUCTION TIME (MINUTES)		TIME (HOURS) OF COAGULATION WHEN INCUBATED AT		PERCENTAGE OF PERSONS DETECTING FORMALDEHYDE BY TASTING THE SAMPLE
	Before adding formaldehyde	After 24 hrs. at 21° C.	Immediately after adding formaldehyde	After 24 hrs. at 21° C.	21° C.	37° C.	
100	18,000	0	‡	‡	X	X	100
250	18,000	0	440	‡	X	X	100
500	18,000	0	24	510	X	X	100
750	18,000	0	22	55	X	X	100
1000	18,000	0	21	22	X	X	100
2500	18,000	0	23	29	X	1296	90
2500	18,000	120	33	38	284	360	80
7500	18,000	460	40	64	188	180	30
10,000	18,000	830	45	125	172	140	50
15,000	18,000	2,000	55	183	188	92	10
20,000	18,000	2,200	72	168	188	48	20
25,000	18,000	77,000	90	140	164	53	10
30,000	18,000	470,000	99	162	116	53	20
40,000	18,000	1,300,000	132	254	130	48	10
50,000	18,000	73,000,000	182	42	100	48	20
75,000	18,000	90,000,000	225	36	124	48	30
100,000	18,000	140,000,000	259	34	116	48	20
0	18,000	120,000,000	660	50	100	48	20

‡ = not reduced in 24 hours.
X = not coagulated after 220 days.

was added to prevent effectively rapid bacterial development, (1:25,000 or more) the reduction time was so greatly shortened that it should arouse suspicion, especially if plate counts or keeping quality tests were also applied. Even the addition of relatively ineffective quantities of formaldehyde (1:30,000 and less) shortened the reduction time to values which were out of harmony with the plate counts.

The tendency for formaldehyde to induce rapid reduction was less pronounced after 24 hours. This suggests that the fall in potential prerequisite to reduction of the dye is delayed either by the removal of part of the formaldehyde added, as a result of interaction with milk constituents, or by the formation of substances which tend more effectively to poise the oxidation-reduction system. However, this does not completely destroy the value of the reduction test as an indicator of the addition of formaldehyde. Within the range of concentrations of formaldehyde most likely to be employed (1:15,000 to 1:25,000) the reduction times after 24 hours were between 183 and 140 minutes although the plate counts were between 2000 and 77,000 per cc. Thus it is evident that the anomalous relationship between the plate counts and reduction time persisted after the milk had been held for 24 hours.

Coagulation time. Two additional sets of milk samples containing the dilutions of formaldehyde indicated in the table were prepared as previously described. One set was placed at 21° C. and the other at 37° C.; these were observed at frequent intervals for the first evidence of coagulation. The data in the table show that concentrations of formaldehyde of 1:25,000 or more markedly delayed coagulation at both temperatures. When formaldehyde in concentrations of 1:1000 or more was added coagulation was indefinitely delayed (more than 220 days).

Detection of formaldehyde by taste. A set of 17 samples containing the concentrations of formaldehyde indicated in the table and 6 control samples containing no formaldehyde were prepared. These 23 samples were arranged and numbered at random. Ten persons tasted these samples and recorded the presence or absence of a formaldehyde taste. Four of the people were actively engaged in the judging of dairy products. The remaining 6 might be classed as ordinary consumers who were not specially trained in the tasting of dairy products.

Before tasting the regular series of numbered samples, each person was required to taste 3 special samples of milk, one of which contained no formaldehyde, one tasted strongly of formaldehyde (1:500), while in the third the formaldehyde was barely detectable by taste. The 10 people who assisted in the tasting were instructed to practice on the special samples until they could differentiate correctly the known samples. All tasting of the unknown samples was done without knowledge or suggestion as to the formaldehyde content and without knowledge of the findings of others who

had tasted the samples. The taste of formaldehyde was reported by some in control samples, especially where a control followed a sample containing a high concentration of formaldehyde. There were many such inconsistencies in the results, due to personal differences and to the carrying over of the taste from one sample to another.

The data in the table show that when formaldehyde was present in concentrations of 1:10,000 or more, it was detected rather consistently by all who tasted the samples of milk. In concentrations of 1:15,000 and less, only 10 to 30 per cent of the tasters detected it. The significance of the ability to detect formaldehyde in very high dilutions is diminished by the fact that 6 of the 10 people reported formaldehyde in 3 of the 6 control samples. Out of the 6 control samples tasted by 10 persons there were 8 cases (13.3 per cent) in which formaldehyde was erroneously reported.

SUMMARY DISCUSSION

It is possible to use the methylene blue reduction test as a basis for suspecting the presence of formaldehyde in milk. The addition of effective quantities of formaldehyde shortens the reduction time, especially if the methylene blue reduction test is run soon after the formaldehyde has been added. It is not intended to imply that the methylene blue reduction test can be used as a specific test for the presence of formaldehyde, but that short reduction times may suggest the application of more exact qualitative tests for the presence of formaldehyde.

The range of dilutions of formaldehyde which effectively retarded bacterial growth, definitely delayed the coagulation time, and at the same time escaped detection by a majority of those who tasted the milk was between 1:15,000, and 1:25,000. The addition of more than 1:15,000 formaldehyde is quite certain to be detected by the consumer, whereas less than 1:25,000 fails to accomplish the purpose for which it is usually added. In other words, the dairyman must be able to adjust the final concentration within this rather narrow range. On a basis of the volume of commercial formalin the margin between "enough" and "too much" is only 0.25 cc. or about 5 drops per gallon. The chemical precision requisite for such careful adjustment is not usually to be expected outside of the laboratory. This suggests that the addition of formaldehyde to milk by the average dairyman is more likely to fail than it is to accomplish the intended result. The addition of too much is likely to be detected by the consumer or to arouse the suspicion of the milk plant operator by anomalous results of the methylene blue reduction test as outlined in this paper. Specifically, any sample of fresh milk which shows a reduction time of less than one hour may be suspected of containing formaldehyde, especially if the keeping quality or the bacterial counts are contraindicative.

PRELIMINARY OBSERVATIONS ON CERTAIN SEASONAL VARIATIONS IN THE PHYSICAL PROPERTIES AND NUTRITIVE VALUE OF COW'S MILK SERUM

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In our work on the problem of zinc in the nutrition of the rat (unpublished data) considerable difficulty was encountered in supplementing the basal synthetic ration with preparations which were potent in the water-soluble vitamin fraction. The basal ration, because of its high purity with respect to zinc, was deficient in all of the water soluble vitamins except B_2, which was sufficiently supplied in 15 per cent of dried egg white. When alcoholic extracts of yeast or liver together with an extract of pork muscle, rich in vitamin B_1, were used to supply the remaining water-soluble factors, poor growth of the animals resulted. It was found that when 2 cc. of whole milk were administered daily to the animals a favorable growth response was obtained. This amount of milk, however, introduced considerable zinc, so an attempt was made to obtain the vitamin B complex from milk and at the same time remove the zinc.

A procedure was developed for the preparation of this fraction from skimmed milk. The skimmed milk was obtained from the University dairy and was an average grade of market milk coming from about twenty herds of cattle. Six gallons of the skimmed milk were placed in a porcelain jar, the temperature brought to 40° C. and 10 cc. of rennet added. After ten minutes the curd was carefully cut and the temperature slowly raised to 46° C., thereby producing a tough curd which settled to the bottom. The resulting whey, which was clear and free from casein particles, was drawn off and allowed to stand at room temperature for 24 hours, permitting the lactic acid organisms to lower the pH to the isoelectric point of the albumin (pH 4.5). It was then placed in a two-liter Erlenmeyer flask and heated in a boiling water bath with frequent stirring until the temperature reached 88° C., which required about 15 minutes. The hot material was cooled on ice and the albumin permitted to settle. The clear, cool serum was siphoned off and concentrated ammonium hydroxide slowly added until a precipitation of the phosphates occurred (pH 9). This precipitate, which contained all but a trace of the remaining zinc, settled to the bottom. The supernatant liquid was withdrawn, and by addition of acetic acid the pH lowered

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to 6. The serum was a clear yellow solution which exhibited a green fluorescence. It had a pleasing odor and taste and was readily consumed by the animals.

About the first of February, 1934, a change had occurred in the milk from which the serum was prepared. At this time it became impossible to obtain a clear yellow fluorescent solution upon the precipitation of the albumin. When ammonium hydroxide was added the resulting phosphate precipitate was obtained with more difficulty and was somewhat slower in settling to the bottom. The serum, when brought to a pH of 6, retained a cloudy appearance in contrast to the clear yellow solution which was obtained before the milk had undergone this change. The milk remained in this condition until about the first week of June, 1934, or until the cows had been placed on pasture. The change to green feed had imparted to the milk some change which allowed the preparation of a clear zinc-low serum.

Another series of experiments was started the first week of October, 1934, with the hope of finishing the experiment before the milk changed to the winter condition. It, however, happened that in this year the milk underwent the change to a winter condition in the last week of October, or about 3 months earlier than in the preceding year. The severe drought of the summer of 1934 had limited very seriously the amount of green pasture for the herds.

The nutrition experiments were continued for 12 weeks on the winter serum. It was therefore possible to compare the growth of the control animals, which had received zinc in the summer serum experiments, with the control animals which received zinc in the winter serum experiments. Since the rations were the same in each case any differences in the rate of growth of the animals could therefore be attributed to the serums from the two seasons.

There were 8 animals on the control ration which received summer milk serum—4 males and 4 females, 9 animals on the winter serum experiment—6 males and 3 females. The animals on the summer serum made an average gain of 111 grams for the first 8 weeks, while those receiving the winter serum gained 89 grams in the same period. See Chart 1.

The fact that the winter milk serum was lower than the summer milk serum in certain nutritional factors was also exhibited in another way. Animals which were placed on the zinc low diet for 6 weeks practically ceased growing. If zinc was then administered there was a strong impulse to grow. Animals on summer milk serum made gains of 16 to 22 grams per week for the 4 weeks following the administration of zinc, while those on winter milk serum made gains of 7 to 12 grams for the same period. See Chart 1. These results are in agreement with those reported by Elvehjem, Hart and others (1), who found that rats on a whole milk diet supple-

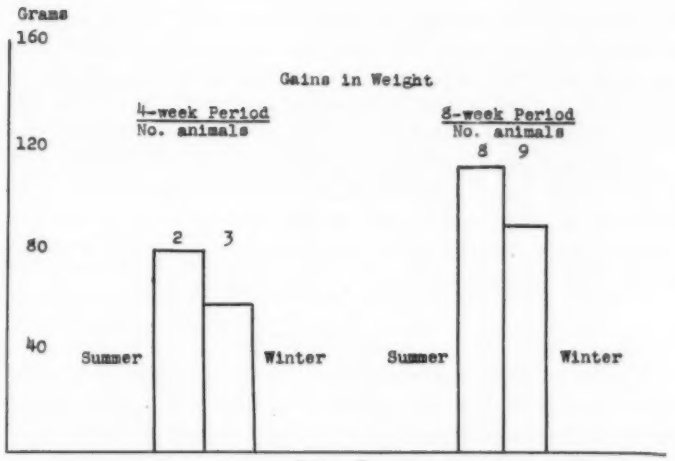

CHART I

Summer and Winter milk serums as supplements to purified rations.

mented with copper, iron, and manganese failed to grow as well on milk produced in the late winter months as on milk produced in the early fall.

Each year when the commercial milks showed the change to the winter condition, milk was obtained from animals of the University herd, which received choice rations of alfalfa hay and corn silage. This milk also changed in its physical behavior at the same time as the commercial milks from the University dairy. Milk from animals receiving artificially dried roughages also exhibited the same physical behavior as the winter commercial milk.

Numerous attempts were made to obtain from these winter milks a clear serum which would resemble that from summer milk. Experiments were made where different salts were added to the whey in varying amounts before the precipitation of the albumin. The salts tried were potassium phosphate (dibasic), sodium citrate, magnesium sulfate, and calcium lactate. One preparation of clear serum was obtained upon the addition of 0.2 gram of calcium lactate per liter of whey. This, however, could not be repeated with the same beneficial effects. It was found later that if the pH of the whey were reduced from 4.5 to 4 by allowing the whey to stand at room temperature for 48 hours instead of 24 and then increased to 4.5 by the addition of ammonium hydroxide, good precipitation resulted. This procedure has not always been found to work satisfactorily for the production of a clear serum from winter produced milk.

It is not possible for us at the present time to state definitely what constituents have been altered in winter milk causing this change in its physical

properties and the decrease in the nutritive value of the serum. The poor growth promoting qualities of winter serum are in accordance with the results obtained when rats are fed mineralized winter milk as the sole diet. This work indicates that the decrease in growth promoting properties of winter milk is due to a deficiency of the water-soluble vitamins in the serum.

It is generally, but not universally, accepted that vitamin B_1 is constant in cow's milk due to its synthesis by intestinal bacteria (2). In respect to B_2 Hunt and Krauss (3) have shown that its level in cow's milk varies with the ration and is highest when the animal is on green pasture. There are no data available in respect to B_4.

SUMMARY

1. The serum of winter produced milk showed physical characteristics at variance with that of summer produced milk. It was clarified with greater difficulty. An adjustment of the salt balance and pH improved somewhat the technique used for the preparation of the serum from winter milk. The time at which these changes occur varies from year to year.

2. The serum of winter produced milk showed lower nutritive value than that of summer produced milk as measured by its use in supplementing a highly purified diet as a source of the vitamin B complex.

3. The significance of these studies lies in the relation of fresh plant tissue as contrasted with field dried material to subtle changes in the milk secreted.

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THE DEVELOPMENT OF NUTRITIONAL ANEMIA IN DAIRY CALVES

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INTRODUCTION

During recent years experimental work has definitely shown that whole milk is not a complete food for certain species of animals. A review of the literature has revealed that the amount of such work reported with calves when this experiment was begun was meager. The experiments reported have shown that attempts to raise calves on milk exclusively have usually ended disastrously. After 6 or more months of such feeding the calves would experience convulsions, finally succumbing during one of the spasms. In view of the strikingly beneficial results obtained with some species through the addition of iron and copper to an exclusive milk diet, it was felt that additional information of scientific interest might be obtained by studying the response of dairy calves to a similar treatment, particularly since whole milk or skimmilk constitutes such a large portion of the ration of calves during the first few months of life.

REVIEW OF LITERATURE

It has long been recognized that whole milk is deficient in iron. The addition of inorganic iron to an exclusive whole-milk diet will not take care of this deficiency in rats (10, 15), pigs (5), or rabbits (10). Later, Hart and coworkers (11) found that, by adding both iron and copper to a whole-milk diet, nutritional anemia in the rat could be prevented or cured. This was found later to apply also to pigs (6).

According to calf feeding experiments (3, 17) in which whole milk was fed as the sole constituent of the diet, malnutrition or death usually ensued after several months of such feeding. Recent experiments indicate that probably this was due to vitamin and mineral deficiencies.

Dairy calves have a definite requirement for vitamin D. Bechdel, Landsburg, and Hill (1), Huffman (12), and Rupel, Bohstedt, and Hart (21) agree that calves will develop rickets on a diet low in vitamin D and can then be cured by supplementing such a diet with vitamin D.

According to Mead and Regan (19) dairy calves have a definite requirement for vitamin A, as well as for certain minerals found in the ash from alfalfa hay. Converse and Meigs (4) also found, by supplementing a poor quality skimmilk diet with cod liver oil, that nearly normal growth resulted without vitamin A deficiencies appearing in the calves.

Cannon (3) found that a whole-milk diet supplemented with cod liver oil and bone meal would furnish sufficient nutrients for dairy calves to an age somewhat over 220 days. After that it was necessary to supply the calves with alfalfa hay or oat straw in order to prevent death from nutritional anemia.

PLAN OF EXPERIMENT

This experiment was conducted with 12 Holstein male calves, divided into two series of six each. All calves were started on the experiment at about 4 days of age.

The first series of six calves was started in 1931 and finished in 1932. The calves were grouped in pairs according to the erythrocyte and hemoglobin content of their blood, as determined when they were 2 days of age. The calf in each pair having the higher erythrocyte count and hemoglobin content was placed in Lot I (Calves M 360, M 363, and M 364), while the calf having the lower erythrocyte count and hemoglobin content was placed in Lot II (Calves M 361, M 362, and M 365).

Whole milk collected at random from a Holstein and Jersey herd was fed twice daily. The maximum amount of milk fed to each calf was regulated according to the consumption of the poorest eater of the series.

Each of the Lot II calves received, in addition to the whole milk, 400 mg. of iron, as ferric chloride, and 40 mg. of copper, as copper sulfate, daily, except Sundays and holidays.

The second series of calves was started in 1933 and finished in 1934. They were divided into Lots I (M 406, M 408, and M 414) and II (M 407, M 411, and M 416) as in the first series, except that the first available calf was placed in Lot I and the next available calf (pair mate) was placed in Lot II, but not on the basis of erythrocyte and hemoglobin values. They were fed whole milk twice daily, at the rate of 1 pound to every 10 pounds of live weight. However, at times some calves would refuse part of their allotted milk. The milk used in this series came from an equal number of Holstein and Jersey cows receiving a ration containing alfalfa hay, corn silage or wet beet pulp, yellow corn, oats, wheat bran, linseed oilmeal, and salt.

The Lot II calves were fed daily, in addition to the whole milk, iron and copper supplements according to live weight; *i.e.*, they were given daily, except Sundays and holidays, 400 mg. of iron, as ferric chloride, and 40 mg. of copper, as copper sulfate, until they weighed 200 pounds. Subsequently, for every 100 pounds additional gain in live weight the iron was increased 200 milligrams and the copper 20 milligrams, so that a calf weighing 200 to 300 pounds received 600 milligrams of iron and 60 milligrams of copper.

The calves were located away from sunshine in the calf barn. Wood

shavings were used as bedding. All calves were muzzled throughout the experiment, except at feeding time. Muzzles made from ½-inch-mesh heavy screen were used in Series I; those used in Series II were made from 0.040-inch pyrolin-viscoloid (a celluloid-like compound). Each pair of calves remained on the experiment until the death of a pair mate or until approximately 8½ months of age. Several times during the experiment all the calves were treated for lice with a 2½ per cent creolin solution.

The number of erythrocytes and the hemoglobin values of the blood were determined when the calves were one and 3 days of age and approximately every 2 weeks thereafter. Histological studies of the erythrocytes were made from time to time. Live weights were obtained at birth and every 2 weeks throughout the experiment. Measurements of the height at withers were made every 2 weeks during the last 3½ months of the first series, and every 2 weeks throughout the experiment during the second series. Post-mortem exeaminations were made. Iron, copper, and vitamin A were determined on the livers of the Series II calves.

METHODS OF ANALYSES

Samples of freely flowing blood for red cell counts were obtained from the median ear vein, by the use of a Trenner automatic diluting pipette. The samples were diluted 200 times with 1 per cent sodium chloride solution. About a drop of this diluted blood was then placed in a Levy-Hauser counting chamber and the number of red cells per cubic millimeter of blood was estimated. Another sample of freely flowing blood for hemoglobin determination was taken and diluted 200 times with approximately 1 per cent hydrochloric acid. After standing for 30 minutes in a warmed room the grams of hemoglobin per 100 cc. of blood were determined in a Bausch and Lomb hemoglobinometer (improved Newcomer model). The condition of the red blood corpuscles was studied by preparing blood films from the calves fed milk exclusively (Series II) when they were very anemic. Similarly, blood cells from their pair mates (fed milk, iron, and copper) were studied at corresponding ages. The blood films were fixed with mercuric chloride and stained with eosin-methylene blue.

Livers taken from the calves in Series II were saved and frozen until a convenient time for analysis for iron, copper, and vitamin A. Iron was determined by the Stugart (22) method and copper by the sodium diethyldithio-carbamate method, according to Williams (23). Vitamin A determinations on the livers were made with the antimony trichloride reagent.

RESULTS

General Health

Series I (1932).—The calves were healthy and vigorous during the first 150 days of the experiment as far as external appearances were concerned.

During the remaining period of 105 days convulsions were frequent, except in calves M 364 (Lot I) and M 361 (Lot II). Scours were very prevalent at times, except in M 364.

External signs of malnutrition were evident toward the latter part of the experiment. There was gradual enlargement of the knees and slight stiffness of the legs resembling rickets. Calves M 363 (Lot I) and M 365 (Lot II) were first to become stiff and emaciated. Respiration was rapid. This occurred when they were about 180 days of age.

All calves but one in this series were alive at the close of the experiment (255 days). M 360 (milk-fed) died at the age of 254 days. This calf appeared healthy on the morning of its death, which occurred during the only convulsion observed in this animal.

Series II (1934).—The calves fed whole milk exclusively (Lot I) appeared healthy externally during the first 120 days of the experiment. For the remaining period of 75 days Calves M 406 and M 414 were rough in the hair coat and scoured very profusely at times. M 406 died when 196 days of age and M 414 was killed on the 194th day because of his extremely poor condition. Convulsions were noted in M 406 at 171 days and 183 days of age. One convulsion was noticed in M 414 at 143 days of age. Calf M 408 remained fairly healthy throughout the experiment except for failing appetite from 162 days of age to the end of the experiment (255 days). All three calves were pale about the muzzle and eyes and rough in the hair coat when they were anemic.

The calves fed milk, iron, and copper (Lot II) remained healthy throughout the experiment, except for two cases of scours, a condition which was prevalent in the general calf herd.

Erythrocyte and Hemoglobin Data

Series 1 and 2 combined.—Results of the erythrocytes and hemoglobin determinations for each pair of calves are given in table 1. The combined average of all determinations of red cell counts for the calves fed whole milk exclusively (Lots I) is 8.59 ± 0.10 millions per cubic millimeter of blood and for the calves fed milk, iron, and copper (Lots II) 9.78 ± 0.09 millions, or a difference of 1,190,000 cells. Owing to a shrunken and broken condition of the erythrocytes (See Figs. 1 and 2) at the time the hemoglobin reached the level of 5 to 6.5 grams, the cell counts are probably unreliable for the milk-fed calves. The same cell condition existed in the milk-fed calves of Series I; however, no complete records were kept. The average amount of hemoglobin per 100 cc. of blood for the exclusive milk-fed calves is 7.62 ± 0.17 grams, which compares very closely with that of "salt sick" yearling cattle (8.38 gm.), as reported by Neal and Becker (20). The average hemoglobin value for calves fed milk, iron, and copper is 10.52 ± 0.10 grams. This lower value, as compared with that for mature

TABLE 1

Red blood corpuscles and hemoglobin data (Calves arranged according to pairs)*

AGE	LOT I		LOT II		LOT I		LOT II		LOT I		LOT II	
	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.	R.B.C. Mil.	Hb. Gm.
SERIES I (1932)												
	M 360		M 361		M 363		M 362		M 364		M 365	
2 days	10.37	13.2	7.94	11.2	14.53	11.0	8.29	8.7	11.57	12.2	6.78	8.1
4 wks.	10.61	9.8	10.38	10.1	8.76	11.6	8.69	10.0	10.75	10.4	9.49	9.8
8 wks.	9.12	10.0	9.65	10.0	7.12	10.0	11.16	11.0	7.81	8.1	9.16	11.0
12 wks.	10.26	9.1	12.90	12.4	10.06	10.1	10.32	11.8	8.17	8.0	12.20	10.3
16 wks.	10.94	9.8	10.56	12.2	10.03	10.1	11.47	12.0	10.11	9.0	11.33	11.5
20 wks.	9.51	5.6	11.90	12.0	8.04	8.9	9.38	14.2	7.58	7.0	11.32	13.4
24 wks.	8.47	5.6	13.26	12.1	7.98	9.2	8.0	9.8	6.82	7.0	10.50	10.2
28 wks.	8.38	7.0	9.98	11.4	8.50	9.5	10.35	13.5	7.35	5.5	10.54	12.6
32 wks.	8.63	8.8	8.72	9.2	7.84	9.0	10.74	11.4	7.34	7.0	9.81	9.9
35 wks.	8.82	7.7	9.04	9.8	8.36	10.5	11.15	12.0	6.52	6.8	8.10	9.5
37 wks.	—	—	7.87	8.5	6.63	8.8	8.78	9.8	6.04	6.3	—	—
SERIES II (1934)												
	M 406		M 407		M 408		M 411		M 414		M 416	
2 days	—	—	8.48	9.7	12.55	12.0	8.26	8.7	8.63	11.4	8.98	11.9
4 wks.	7.66	8.8	10.34	10.0	10.70	13.0	10.50	10.2	8.69	5.8	10.09	12.6
8 wks.	8.48	6.4	8.74	12.0	11.81	10.5	11.86	9.8	8.75	6.3	8.35	10.5
12 wks.	9.12	4.8	10.27	11.5	12.43	8.8	10.23	12.0	7.70	5.5	9.01	8.8
16 wks.	8.71	5.3	9.73	9.3	8.90	7.8	8.93	9.5	5.88	3.4	8.23	8.6
20 wks.	9.09	4.0	9.10	9.7	8.93	6.5	8.35	8.7	5.23	3.3	7.16	8.3
24 wks.	7.87	3.1	8.95	10.8	8.13	4.5	8.71	8.4	6.10	3.2	7.19	9.3
28 wks.	9.18	4.1	7.62	8.8	6.97	4.0	7.89	8.5	9.44	4.1	9.20	7.3
32 wks.	—	—	—	—	6.93	3.4	6.64	8.0	—	—	—	—
37 wks.	—	—	—	—	6.44	4.5	7.19	8.0	—	—	—	—

* The Lot I calves received whole milk exclusively and the Lot II calves received whole milk with iron and copper supplements.

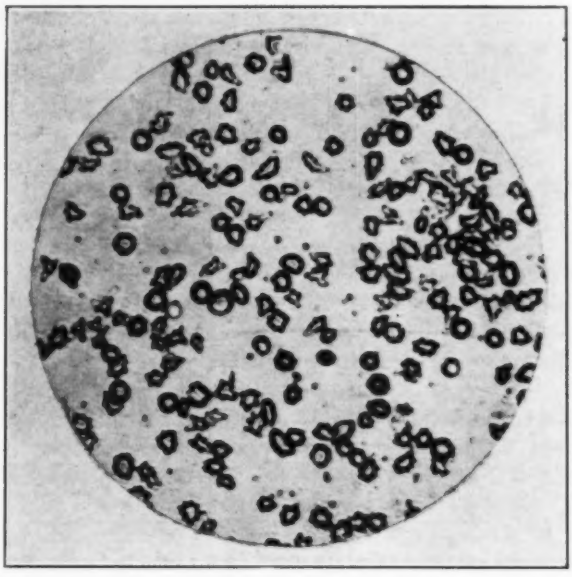

FIG. 1. PHOTOMICROGRAPH OF A BLOOD SMEAR FROM A MILK-FED CALF OF SERIES II SHOWING CONDITION OF RED CELLS AT TIME OF SEVERE ANEMIA (×550).

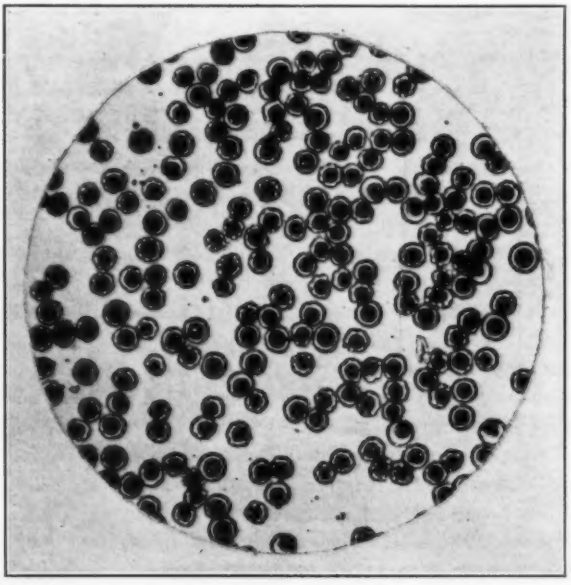

FIG. 2. PHOTOMICROGRAPH OF A BLOOD SMEAR FROM A CALF FED MILK, IRON, AND COPPER OF SERIES II (PAIR MATE TO ABOVE) SHOWING CONDITION OF RED CELLS (×550).

bulls of 11.92 ± 0.26 gm. (2) and 12.8 ± 0.8 gm. (18), indicates that the concentration of hemoglobin in the blood increases as the rate of growth decreases with advancing age. Table 1 shows that the hemoglobin values for the calves of Lots II increased soon after birth and then gradually decreased with advancing age, indicating that as the rate of growth increases the concentration of hemoglobin tends to decrease.

GROWTH

The average rate of gain in live weight for the Lot I and II calves in Series I was practically the same for the first 224 days. During the remaining 31 days those in Lot I decreased slightly in live weight, while those receiving whole milk, iron, and copper (Lot II) continued to increase in weight. The average live weight at the close of the experiment (255 days) for the Lot I calves was 363 pounds and for the Lot II calves 430 pounds. A similar situation was found in the second series. Average live-weight gains were about the same for both lots until they were 112 days of age. At about 196 days of age the Lot I calves averaged 353 pounds; whereas the Lot II calves averaged 517 pounds.

TABLE 2
Weights of organs and color of tissues

CALF NO.	WT. OF TESTES GM.	WT. OF SPLEEN GM.	WT. OF LIVER GM.	WT. OF HEART GM.	COLOR OF LIVER	COLOR OF MUSCLE TISSUE
			Lot I*			
M 360	91	589	1973	885	Very pale	Very pale
M 363	91	329	2086	748	Slightly pale	Slightly pale
M 364	159	476	2472	839	Pale	Pale
M 406	91	181	2403	997	Very pale	Very pale
M 408	227	612	3809	1723	Pale	Pale
M 414	45	227	3084	907	Pale	Pale
Average	117	402	2638	1016		
			Lot II*			
M 361	238	749	2222	1089	Dark red	Dark red
M 362	227	635	2086	816	Dark red	Red
M 365	181	340	1587	748	Dark red	Red
M 407	159	1088	5442	1406	Dark red	Dark red
M 411	281	862	5669	1723	Dark red	Dark red
M 416	141	794	2648	1224	Red	Red
Average	204	744	3275	1167		
Per cent Diff...	42.6	45.9	19.4	12.9		

* Lot I received whole milk exclusively and Lot II received whole milk with iron and copper supplements.

A slower rate of growth in height at the withers was found in the exclusive milk-fed calves as compared with the calves fed milk and supplement, especially during the latter period of the experiment.

Post-Mortem Findings

The results of post-morten examinations are given in table 2.

Differences in total body weight between the anemic and non-anemic calves preclude a direct comparison of the effect of iron and copper on the development of body organs. However, there was a marked and consistent difference in the size of the testes and spleen, those of the calves fed iron and copper being much larger.

The kidneys appeared normal in every respect.

The iron and copper content of the calf livers of Series II (Table 3) substantiate the work of others (8, 9) in demonstrating that the liver acts

TABLE 3

Iron and copper content of livers

SERIES II

CALF NO.	WEIGHT GM.	IRON PCT.	TOTAL IRON MG.	COPPER PCT.	TOTAL COPPER MG.
			LOT I		
M 406	2403	0.00366	87.9	0.00122	29.3
M 408	3809	0.00241	91.8	0.00087	33.1
M 414	3084	0.00308	94.9	0.00232	71.5
Av.	3099	0.00295	91.5	0.00144	34.6
			LOT II		
M 407	5442	0.00715	389.1	0.00992	539.8
M 411	5669	0.00752	426.3	0.01347	763.6
M 416	2648	0.00992	262.7	0.01305	345.5
Av.	4586	0.00783	359.3	0.01198	549.6

as a storehouse for iron and copper. The iron values given compare favorably with those reported by Elvehjem and Peterson (7), but, according to the copper values reported by Lindow, Elvehjem, and Peterson (16), the calves fed iron and copper were receiving a considerable excess of copper.

GENERAL DISCUSSION

The hemoglobin values given in table 1 indicate that the exclusive milk-fed calves (Series I), as compared with the calves fed milk, iron and copper were getting enough iron from some source to effect an increase in the hemoglobin content of their blood, especially during the last 3 months of the experiment. Considerable trouble was encountered throughout this series in keeping all the calves from eating shavings. At times they would tear

or wear holes in their muzzles, allowing them to eat wood shavings or lick the iron stalls. Towards the latter part of the experiment the calves fed whole milk exclusively (Lot I) required new muzzles about every 2 or 3 weeks. The calves in Series II were practically unable to eat any foreign material because of the new type of muzzle made from 0.040-inch pyrolin-viscoloid. We feel that the use of a satisfactory muzzle in Series II was largely responsible for the more uniform results obtained.

The rate of growth of the first series, as compared with the second series, indicates that the system of feeding was inadequate in Series I in that it did not supply the calves with sufficient nutrients for rapid growth. The limiting factor may have been not only a shortage of total nutrients, as pointed out by Hughes and Cave (13), but an inadequate supply of vitamin D, as indicated by calcium and phosphorus determinations of the blood serum, and probably a deficiency of manganese, as indicated by Kemmerer, Elvehjem, Hart, and Fargo (14). The rate of growth of the calves fed iron and copper in Series II was, with one exception (M 416), very rapid throughout the experiment. M 416 grew very slowly for the first 4½ months but very fast during the remaining period of the experiment. The thyroid gland from this calf weighed 73 grams (2.7 times heavier than the average weight of thyroids taken from the other five calves in Series II), indicating iodine deficiency at birth.

No external symptoms of vitamin A deficiency were noticed in any of the calves, even though the vitamin A content of the livers from the last series of calves was about one-seventh the amount found in livers from normal calves of the same age.

SUMMARY

Six pairs of Holstein male calves were used in a study of the susceptibility of calves to nutritional anemia when fed whole milk exclusively and of the effect of supplementing such a diet with inorganic iron and copper.

Physical appearance, growth, determinations of the number of erythrocytes and amount of hemoglobin in the blood, and post-mortem examination showed that the calves on milk alone developed nutritional anemia while those receiving iron and copper in addition to milk did not.

Considerable difference in appearance and size of the internal organs was revealed on post-mortem examination. Of particular interest in this connection was the marked difference in size of the testes and spleens, those of the calves receiving the mineral supplements being much larger.

The livers of the calves fed iron and copper were found to contain large quantities of these two minerals as compared to the amounts found in the livers of calves fed milk exclusively.

CONCLUSIONS

Holstein male calves develop nutritional anemia when fed whole milk exclusively.

The daily addition of 400 mg. or more of inorganic iron and 40 mg. or more of inorganic copper to an exclusive whole-milk diet will prevent the development of nutritional anemia in calves.

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American Dairy Science Association Announcements

Housing Plans for the 1935 Meeting

The School of Agriculture dormitories will be available to those attending the American Dairy Science meeting at University Farm, St. Paul. The rooms are large, airy and well lighted and it shall be the aim of the committee on housing to see that everyone is comfortable and happy while here, at a cost of 75 cents per person per night. The members of the committee on housing and registration are Superintendent J. O. Christianson and Dr. W. E. Peterson at University Farm.

If you wish to bring the family, there are several suites where accommodations may be arranged. However, it is very important that all reservations be made as early as possible. The postcards which the members returned to the Program Committee were intended only as a general guide. *Those who indicated that they expect to make reservations at the dormitories should not regard such indication as an actual reservation.* This must be made directly with the chairman of the housing committee. *Send reservations to Superintendent J. O. Christianson, University Farm, St. Paul, Minn.*, and state carefully the number of persons in your party, men, women and children.

Reduced Railroad Fares

Reduced fares will be in effect from all points for this meeting, available as "Convention Fares" of a fare and one third for the round trip. Certain territories will have very low "Summer Tourist" fares, and "Summer Tourist Short Limit" tickets on sale, that will provide an opportunity to extend your trip, if desired. Consult your local railroad ticket agent for cheapest fares from your particular location, and he will advise you regarding cost and possible extensions of trip beyond St. Paul.

Program of Entertainment for the Ladies

June 24—Monday

8:30 p. m.—Informal social get-together for members and their families, Home Economics Building, University Farm.

June 25—Tuesday. Hosts for the day—Twin City (St. Paul and Minneapolis) Dairy Industry.

9:00 a. m.–12:30 p. m. Sight-seeing automobile trip of the Twin Cities.

12:45 p. m.—Complimentary luncheon, Radisson Inn, Lake Minnetonka.

2:30 p. m.—Return to University Farm Campus.

4:00 p. m.—Members and their families leave for trip to Land-O-Lakes Creameries, Inc.

6:00 p. m.—Complimentary dinner and entertainment as guests of Land-O-Lakes Creameries, Inc.

June 26—Wednesday. Host for the day—General Milk Inc.

9:00 a. m.—Busses will call for the ladies at University Farm and conduct them on a tour of the University of Minnesota Campus, Minneapolis, and of the Washburn Crosby Flour Mills.

12:00 noon—Complimentary luncheon at Nicollet Hotel, Minneapolis.

2:30 p. m.—Return by busses to University Farm, St. Paul.

4:00 p. m.—Tea, Fireplace Room, Home Economics Building, University Farm. Mrs. Walter C. Coffey, *Hostess.*

5:00 p. m.—Leave for Minneapolis Automobile Club.

7:00 p. m.—Subscription banquet and entertainment at Minneapolis Automobile Club.

June 27—Thursday. Shop, golf or other optional activities; or merely rest.

Suggestions for Presentation of Papers

The Society of American Bacteriologists and the American Chemical Society have recently published the following suggestions to their members for the purpose of increasing the effectiveness of their scientific programs. These suggestions are so clear cut and pointed that they are well worth passing on to the members of our association who may not have seen them.

"1. Arrangement of Material. Manuscripts as prepared for publication are seldom suitable for oral presentation. The paper should convey clearly to the hearer: (a) the purpose of the work; (b) the experimental method; (c) the results obtained; and (d) conclusions. The nature of the material and the time available for presentation will determine the degree of emphasis to be placed on each subdivision. The author should make certain by trial against his watch that the essential points can be adequately presented in the time allotted to the paper."

"2. Statement of Purpose. Orient the audience clearly as to the nature and purpose of the work. A lengthy historical review is generally out of place."

"3. Technic. Describe the experimental method employed, so as to indicate the principles involved. Omit details of apparatus or procedure unless there is some particularly novel development. Such data may belong in the published paper but will bore your audience."

"4. Statement of Results. Present the results graphically, preferably with diagrams. Lantern slides are more clearly seen than hand-drawn charts. These slides should be of standard size (3.25 x 4 inches) and should project clearly on the screen. Regardless of who has made the charts or slides, try them from the point of view of the audience

before presenting them at the meeting. Do not read tables, a procedure which wastes time and destroys interest, but point out the general trend of the data."

"5. Conclusions. Summarize the evidence and discuss the importance of the results or conclusions to the particular field of research involved."

"6. Manner of Presentation. Do not read from a manuscript verbatim. Talk directly to your audience in a clear, loud voice. Do not face blackboard or screen while speaking. Articulate distinctly."

"7. Many exceptions to, and modifications of, the above suggestions will apply in particular instances. Nevertheless, general adherence to the points brought out will go far in eliminating the valid criticisms which have been aimed at our programs."

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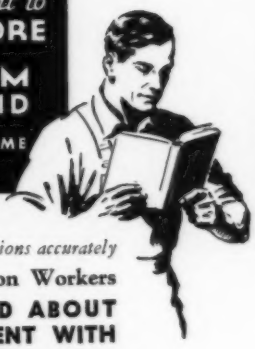

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